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UNIVERSITY OF ALBERTA

DETERMINATION OF MAGNESIUM ION IN COMPLEX SOLUTIONS USING IMMOBILIZED LIGANDS

BY

TODD RICHARD DEMONE

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta
SPRING 1996

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This is dedicated to my mother, Carla, who will always be my inspiration.

ABSTRACT

The viability of a method for speciation studies involves consideration of several factors. The method must be selective for the desired species; the method of detection must also be selective for the desired element; the determined values must be analytically useful over the desired range of species concentrations, and; the method of analysis must not perturb the test system.

The column equilibration method is used to perform this investigation. Complexing ligands that have been immobilized onto solid supports are investigated for their species selectivity and their linear range response capability. The selectivity of the detection is accomplished by use of the element-selective technique of atomic absorption spectrophotometry. The non-perturbing condition requires that, at equilibrium, only a very small amount (<1%) of the immobilized ligand's complexing capacity is occupied. This is accomplished by utilizing ligands having small formation constants with the desired species.

The solid supports used in this study consist of controlled pore glass (CPG) and polymer (P) beads. The ligands immobilized onto these supports include oxine, phenanthroline, phenylglycine and picolinic acid. Factors such as the nature of the solid support, the capacity of the immobilized ligand and the complexation constant between the immobilized ligand and the desired species are investigated.

The resulting low capacity of the CPG-bound ligands gives very small differences between the complexed Mg^{2+} and Mg^{2+} which exists in solution in the column. In order to minimize the effect of the non-complexed Mg^{2+}, higher capacity P-bound ligands are used.

The high capacity, low complexation materials result in a response that is linear up to about the mM level of Mg^{2+} in solution. This value is significant in that it is the range for Mg^{2+} that occurs in biological fluids.

The immobilized ligand, P-Picolinic Acid, was successfully found to give a linear response up to 0.5 mM and the system was selective for the Mg^{2+} species in the presence of solutions containing positive, neutral and negatively charged Mg-ligand complexes.

TABLE OF CONTENTS

PAGE

CHAPTER ONE

INTRODUCTION

1.1 IMPORTANCE OF MAGNESIUM....1
1.2 COLUMN EQUILIBRATION TECHNIQUE....2
1.3 MAGNESIUM SELECTIVE ELECTRODES....6
1.4 MAGNESIUM INDICATORS....7
1.5 ^{31}P NMR....8
1.6 OTHER METHODS....9

CHAPTER TWO

EXPERIMENTAL

2.1 CHEMICALS, SOLVENTS AND GASES....11
2.2 PREPARATION OF METAL SOLUTIONS....12
2.3 SYNTHESIS OF IMMOBILIZED LIGANDS....12
2.3.1 CPG-OXINE....12
2.3.2 CPG-PHENANTHROLINE....14
2.3.2.1 PREPARATION OF 5-AMINO-1,10-PHENANTHROLINE....14
2.3.2.2 PREPARATION OF CPG/ALDEHYDE DERIVATIVE....14
2.3.2.3 IMMOBILIZATION OF LIGAND ONTO SUPPORT....14
2.3.3 P-PHENANTHROLINE....16
2.3.3.1 MODIFICATION OF POLYMER BEADS....16
2.3.3.2 IMMOBILIZATION OF LIGAND ONTO SUPPORT....16
2.3.4 P-PHENYLGLYCINE....16
2.3.4.1 MODIFICATION OF POLYMER BEADS....16
2.3.4.2 IMMOBILIZATION OF LIGAND ONTO SUPPORT....19
2.3.5 P-PICOLINIC ACID....19
2.3.5.1 PREPARATION OF PICOLINIC ACID....19
2.3.5.2 MODIFICATION OF POLYMER BEADS....21
2.3.5.3 IMMOBILIZATION OF LIGAND ONTO SUPPORT....21
2.4 EQUIPMENT....22

2.5 COLUMN EQUILIBRATION APPARATUS....23
2.6 ANALYTICAL COLUMN....26
2.7 COLUMN EQUILIBRATION PROCEDURE....28
2.8 ELUTION CONDITIONS....29
2.9 CAPACITY STUDY....32
2.9.1 WASHING CONDITIONS IN CAPACITY STUDY....33
2.9.2 LOADING CONDITIONS IN CAPACITY STUDY....33
2.9.3 COPPER SORPTION ISOTHERM IN CAPACITY STUDY....36

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Mg^{2+} SORPTION PROPERTIES....41
3.1.1 CPG-OXINE....44
3.1.1.1 LOADING CURVE....44
3.1.1.2 ISOTHERM....44
3.1.1.3 RECOMMENDATIONS....47
3.1.2 CPG-PHENANTHROLINE....47
3.1.2.1 LOADING CURVE....47
3.1.2.2 ISOTHERM AND HOLD-UP VOLUME....49
3.1.2.3 RECOMMENDATIONS....52
3.1.3 P-PHENANTHROLINE....52
3.1.3.1 LOADING CURVE....52
3.1.3.2 ISOTHERM....54
3.1.3.3 RECOMMENDATIONS....57
3.1.4 P-PHENYLGLYCINE....58
3.1.4.1 LOADING CURVE....58
3.1.4.2 PRECONDITIONING STUDY....58
3.1.4.3 FILTERED LOAD STUDY....63
3.1.4.4 ISOTHERM....65
3.1.4.5 RECOMMENDATIONS....65
3.1.5 P-PICOLINIC ACID....68
3.1.5.1 LOADING CURVE....68
3.1.5.2 TYPICAL CALIBRATION CURVE....68
3.1.5.3 ISOTHERM....71
3.1.5.4 RECOMMENDATIONS....71

3.1.6 SUMMARY....71
3.2 SPECIFICITY OF IMMOBILIZED LIGANDS FOR Mg^{2+}....74
3.2.1 EDTA AS SOLUBLE LIGAND....75
3.2.1.1 P-PICOLINIC ACID AS IMMOBILIZED LIGAND....82
3.2.1.2 P-PHENYLGLYCINE AS IMMOBILIZED LIGAND....82
3.2.2 PICOLINIC ACID AS SOLUBLE LIGAND....86
3.2.2.1 P-PICOLINIC ACID AS IMMOBILIZED LIGAND....89
3.2.2.2 P-PHENYLGLYCINE AS IMMOBILIZED LIGAND....90
3.2.3 OXALIC ACID AS SOLUBLE LIGAND....94
3.2.3.1 P-PICOLINIC ACID AS IMMOBILIZED LIGAND....97
3.2.4 SPECIFICITY SUMMARY....100
3.3 ADDITIONAL STUDIES....101
3.3.1 EFFECT OF IONIC STRENGTH....101
3.3.2 EFFECT OF pH....103
3.3.3 SUMMARY....105

LITERATURE CITED....107

APPENDIX....111
a) Data tables for the figures in the thesis....112
b) Computer programs used in the specificity studies....126

LIST OF TABLES

PAGE

Table 2.1. Elution conditions for CPG-Oxine.....31

Table 2.2. Elution conditions for P-Phenanthroline.....31

Table 2.3. Summary of elution conditions for the immobilized ligands.....31

Table 2.4. Capacity values for the immobilized ligands.....38

Table 3.1. Formation constants for the immobilized ligands.....40

Table 3.2. (a) Top - Protonation values for EDTA
(b) Bottom - Complexation values for Mg-EDTA.....80

Table 3.3. (a) Top - Mg^{2+} specificity in the presence of Mg-EDTA complexes using the P-Picolinic acid system
(b) Bottom - Mg^{2+} specificity in the presence of Mg-EDTA complexes using the P-Phenylglycine system.....84

Table 3.4. (a) Top - Protonation values for Picolinic acid
(b) Bottom - Complexation values for Mg-Picolinic acid.....88

Table 3.5. (a) Top - Mg^{2+} specificity in the presence of Mg-Picolinic acid complexes using the P-Picolinic acid system
(b) Bottom - Mg^{2+} specificity in the presence of Mg-Picolinic acid complexes using the P-Phenylglycine system.....92

Table 3.6. (a) Top - Protonation values for Oxalic acid
(b) Bottom - Complexation values for Mg-Oxalic acid.....96

Table 3.7. Mg^{2+} specificity in the presence of Mg-Oxalic acid complexes using the P-picolinic acid system.....98

Table A1. Cu^{2+} WASH STUDY : CPG-PHENANTHROLINE.....112

Table A2. Cu^{2+} LOAD STUDY : CPG-PHENANTHROLINE.....112

Table A3. Cu^{2+} CONCENTRATION STUDY : CPG-PHENANTHROLINE.....113

Table A4. LOAD STUDY : CPG-OXINE.....113

Table A5. ISOTHERM STUDY : CPG-OXINE.....114

Table A6. LOAD STUDY : CPG-PHENANTHROLINE.....114

Table A7. ISOTHERM AND BLANK STUDY : CPG-PHENANTHROLINE.....115
Table A8. ISOTHERM STUDY : CPG-PHENANTHROLINE.....115
Table A9. LOAD STUDY : P-PHENANTHROLINE.....116
Table A10. ISOTHERM AND BLANK STUDY : P-PHENANTHROLINE.....116
Table A11. ISOTHERM STUDY : P-PHENANTHROLINE.....117
Table A12. LOAD STUDY : P-PHENYLGLYCINE.....117
Table A13. PRECONDITIONING LOAD STUDY : P-PHENYLGLYCINE.....118
Table A14. FILTERED PRECONDITIONING STUDY : P-PHENYLGLYCINE.....118
Table A15. LOAD STUDY : P-PHENYLGLYCINE.....119
Table A16. ISOTHERM AND BLANK STUDY : P-PHENYLGLYCINE.....119
Table A17. ISOTHERM STUDY : P-PHENYLGLYCINE.....120
Table A18. LOAD STUDY : P-PICOLINIC ACID.....120
Table A19. TYPICAL CALIBRATION CURVE.....121
Table A20. ISOTHERM STUDY : P-PICOLINIC ACID.....121
Table A21. Mg-EDTA SPECIFICITY STUDY : P-PICOLINIC ACID.....122
Table A22. Mg-EDTA SPECIFICITY STUDY : P-PHENYLGLYCINE.....122
Table A23. Mg-PICOLINIC ACID SPECIFICITY : P-PICOLINIC ACID.....123
Table A24. Mg-PICOLINIC ACID SPECIFICITY : P-PHENYLGLYCINE.....123
Table A25. Mg-OXALIC ACID SPECIFICITY : P-PICOLINIC ACID.....124
Table A26. IONIC STRENGTH STUDY : P-PICOLINIC ACID.....124
Table A27. pH STUDY : P-PICOLINIC ACID.....125

LIST OF FIGURES

PAGE

Figure 1.1. A representation of a breakthrough curve....4

Figure 2.1. The structure of immobilized CPG-Oxine....13
Figure 2.2. A schematic of the preparation of CPG-Phenanthroline....15
Figure 2.3. A schematic of the preparation of P-Phenanthroline....17
Figure 2.4. A schematic of the preparation of P-Phenylglycine....18
Figure 2.5. A schematic of the preparation of P-Picolinic acid....20
Figure 2.6. Direct elution into the atomic absorption spectrophotometer....24
Figure 2.7. Collection of eluted sample....25
Figure 2.8. Top - A schematic of the slider contained in V3.
Bottom - An enlargement of the column (middle hole)....27
Figure 2.9. WASH STUDY : CPG-PHENANTHROLINE....34
Figure 2.10. LOAD STUDY : CPG-PHENANTHROLINE....36
Figure 2.11. COPPER SORPTION ISOTHERM : CPG-PHENANTHROLINE....37

Figure 3.1. LOADING CURVE : CPG-OXINE....45
Figure 3.2. ISOTHERM : CPG-OXINE....46
Figure 3.3. LOADING CURVE : CPG-PHENANTHROLINE....48
Figure 3.4. ISOTHERM / BLANK : CPG-PHENANTHROLINE....50
Figure 3.5. ISOTHERM : CPG-PHENANTHROLINE....51
Figure 3.6. LOADING CURVE : P-PHENANTHROLINE....53
Figure 3.7. ISOTHERM / BLANK : P-PHENANTHROLINE....55
Figure 3.8. ISOTHERM : P-PHENANTHROLINE....56
Figure 3.9. LOADING CURVE : P-PHENYLGLYCINE....59
Figure 3.10. PRECONDITIONING LOADING CURVE : P-PHENYLGLYCINE....61

Figure 3.11. FILTERED PRECONDITIONING LOAD : P-PHENYLGLYCINE.....62
Figure 3.12. FILTERED LOADING CURVE : P-PHENYLGLYCINE.....64
Figure 3.13. ISOTHERM / BLANK : P-PHENYLGLYCINE.....66
Figure 3.14. ISOTHERM : P-PHENYLGLYCINE.....67
Figure 3.15. LOADING CURVE : P-PICOLINIC ACID.....69
Figure 3.16. TYPICAL CALIBRATION CURVE.....70
Figure 3.17. ISOTHERM : P-PICOLINIC ACID.....72
Figure 3.18. EDTA SPECIFICITY : P-PICOLINIC ACID.....83
Figure 3.19. EDTA SPECIFICITY : P-PHENYLGLYCINE.....85
Figure 3.20. PICOLINIC ACID SPECIFICITY : P-PICOLINIC ACID.....91
Figure 3.21. PICOLINIC ACID SPECIFICITY : P-PHENYLGLYCINE.....93
Figure 3.22. OXALIC ACID SPECIFICITY : P-PICOLINIC ACID.....99
Figure 3.23. EFFECT OF IONIC STRENGTH : P-PICOLINIC ACID.....102
Figure 3.24. EFFECT OF pH : P-PICOLINIC ACID.....104

CHAPTER ONE

INTRODUCTION

A knowledge of the species distribution of metal ions in complex systems is very useful. The amount of metal ion in a sample can be characterized analytically by either the total amount of metal present or by the quantitation of the different chemical forms of the metal. These forms consist of metal which is uncomplexed and that which is complexed to various inorganic and organic ligands. Uncomplexed metal ion, M^{n+}, refers to metal ion that exists in solution within a hydration sphere. The metal ion is surrounded by water molecules and is referred to as the "free" species. The complexes that form with metal ions can be referred to as kinetically labile or inert. Inert refers to a complex that remains intact whereas kinetically labile species have the ability to interconvert rapidly with one another.

The importance of the speciation of metals is evident in situations where only certain forms have a relevant role. For example, higher levels of copper in water are permitted and satisfy water quality standards where organic complexation is present since it is the uncomplexed metal ion which is the toxic form [1]. In this report, the metal of interest is magnesium.

1.1 IMPORTANCE OF MAGNESIUM

Magnesium is the fourth most abundant cation in the human body and the second most plentiful in body cells [2]. The analysis of magnesium has been limited to the total concentration of the element since there is a lack of a

sensitive method to measure only the ionized fraction. For example, the total concentration of magnesium in blood serum ranges between 0.6 and 1 mmol/L whereas the range of ionized Mg^{2+} can only be estimated to be between 0.45 and 0.8 mmol/L [3,4].

The magnesium ion is known to have an important function in the human body. This divalent cation is a cofactor of many enzymes acting on adenosine triphosphate (ATP) and it plays an essential role in the regulation of cellular permeability and neuromuscular excitability [5]. It is also known that changes in the intracellular Mg^{2+} concentration have critical effects on cellular metabolism, structure and bioenergistics [6]. Magnesium ions also mediate DNA synthesis, hormone releases and the transport of different ions across various membranes [7]. However, the exact correlation between these functions and the levels of magnesium ions has not been fully realized because of the need for direct measurement of the concentration of the Mg^{2+} species.

1.2 COLUMN EQUILIBRATION TECHNIQUE

A metal may be involved in several metal-ligand equilibria and may be present in many kinetically labile species in systems such as biological fluids. In order to achieve an accurate determination of a certain species in such a system, the method of determination should be non-perturbing. This requirement forms the basis of the column equilibration technique.

The column equilibration technique has previously been used to investigate the free nickel ion concentration in sewage [8], the free copper ion concentration in natural waters [9], the free calcium ion concentration in

aqueous solutions [10] and the free magnesium ion concentration in aqueous solutions [11]. This technique involves pumping a fresh sample solution through a bed of resin until the resin has come to equilibrium with the sample solution. At this time, the resin is no longer removing the desired species from the sample solution. Therefore, no perturbing of the sample solution is occurring since the composition of sample solution is not changing.

There are some characteristics and terminology of the column equilibration technique that need to be introduced. Pumping a sample solution through a bed of resin is referred to as the **loading step.** A plot of concentration in the effluent versus time or volume that a sample flows through the resin bed during the loading step is a **breakthrough curve.** In Figure 1.1 the ratio of the effluent concentration (that which exits the column), C_E, to the influent concentration (that which enters the column), C_I, is plotted against the time or volume. The time for the ratio of C_E/C_I to reach unity - where the curve levels off - is referred to as the **equilibrium loading time.**

The **distribution coefficient,** λ_0, relates the amount of the metal ion (MR) sorbed by the resin to the concentration of free metal ion (M^{n+}) in solution.

$$\lambda_0 = \frac{[MR]}{[M^{n+}]}$$

A plot of [MR] versus $[M^{n+}]$ is an **isotherm.** In order for this relation to be linear and λ_0 be a constant, **trace loading conditions** must be maintained. This means that the amount of metal ion sorbed, [MR], must be much less than the total available capacity of the resin.

$$[MR] << [R] + [HR] + [MR]$$

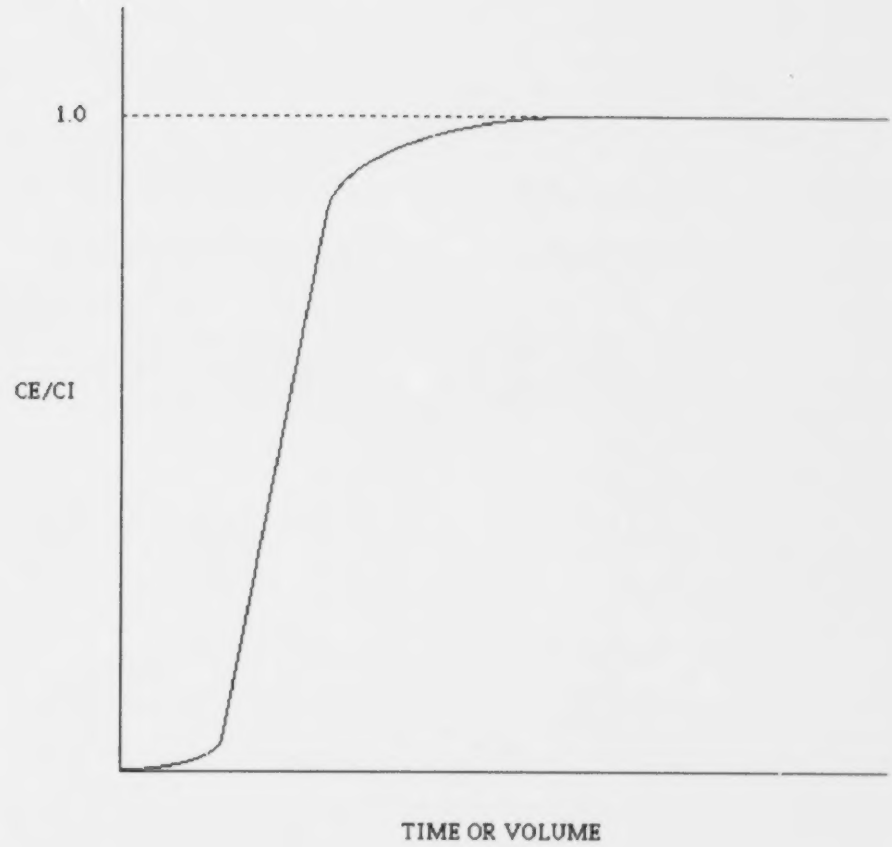


Figure 1.1. A representation of a breakthrough curve.

This value should be less than 1 percent. The **linear sample capacity** can be measured by determining the range of [MR] over which λ_0 is constant.

The selection of the resin for these types of studies is obviously very important. The trace loading condition stipulates that only a small fraction of the complexing ligand of the resin can be complexed by the metal ion at equilibrium. This means that the **formation constant** between the ligand and the metal ion must be very small. An ion exchange method studied by Hewavitharana and Kratochvil [12] successfully determined the concentration of Mg^{2+} ions in the presence of magnesium-ligand complexes. However, this result was only valid at the μM level. In order to be used at higher levels (i.e. mM), a high concentration of swamping electrolyte had to be added to the system in order for trace conditions of ion exchange to be maintained. That is, so only about 1% of the available ion exchange sites would be occupied by the Mg^{2+} species. This elevated ionic strength resulted in changes to the distribution in the sample solution and therefore, the application of this method at higher Mg^{2+} ion concentrations was invalid.

Therefore, control over the complexing nature of the resin is required for this technique. The immobilization of selected ligands onto a solid support best satisfies this situation. It is true that ligands tend to form complexes with more than one metal ion. This lack of element specificity is eliminated by using the element-specific detection method of **atomic absorption spectrophotometry**. An important source of interference in complicated solutions that has to be considered in the column equilibration technique is the sorption of metal-ligand complexes onto the resin. The signal obtained from the AAS in such cases would be a combination of the desired uncomplexed metal ion and the unwanted metal-ligand complex. This is an important

consideration when assessing the selectivity of the resin for the free metal ion species.

Chapters 2 and 3 of this report examine various immobilized ligands and solid supports and determines the viability of these systems for the analysis of uncomplexed Mg^{2+} ions in the presence of its metal-ligand complexes. There are examples in the literature of other methods that are being investigated in an attempt to accurately characterize complex magnesium systems. A brief description of these methods is given in the next section.

1.3 MAGNESIUM SELECTIVE ELECTRODES

The conventional way of determining the pH of a solution is to use a glass electrode which measures the hydrogen ion activity. Based on this well-known and established example, it makes sense that a similar methodology would be considered for the measurement of the magnesium ion in solution. Electrodes are electrochemical sensors that allow the potentiometric determination of the activity of certain ions in the presence of other ions.

There have been several examples in the literature over the last decade of attempts at fabricating a satisfactory magnesium ion-selective electrode [13-16]. Most of this work has come from the laboratory of the late Professor Wilhelm Simon of the Swiss Federal Institute of Technology [3,4,17-21]. Designs based on neutral carriers have suffered from heavy interference from hydrogen ions [17] and calcium ions [3,4,18,19]. Also, in working with real samples, these electrodes have shortcomings such as irregular responses [3], electrical asymmetry at their surfaces and reduced lifetimes [4]. Presently, one of these electrodes is being used in a commercial instrument [2,22].

However, the analysis involves well-matched standard solutions for calibration and measurement and correction for calcium ion interference.

1.4 MAGNESIUM INDICATORS

Fluorescent dyes are being used to determine the magnesium ion concentration in solutions. The clinical applications include the measurement of the intracellular concentration of the uncomplexed ion using such indicators as mag-fura-2, mag-indo-1 [23-26] and mag-fura-5 [7]. These dyes are incubated with the test cells, allowing time for the dyes to enter the cell and complex the Mg^{2+} ions. The fluorescence signal of the complex is then measured and correction is made for the contribution of the uncomplexed indicator. These dyes have a high binding affinity for free magnesium ions. As a result of the dye molecules entering the cells and complexing with Mg^{2+} ions, the numerous equilibria are shifted. That is, the dyes perturb the equilibria. The signals obtained are therefore related to the new Mg^{2+} ion concentration and not the original, desired values. These types of dyes are typically very expensive and special laboratory handling procedures are required.

The determination of Mg^{2+} ions in extracellular fluids such as serum and urine and in bulk test solutions has also been studied using indicator dyes. Again, the method involves the formation of a complex which is then analyzed for its fluorescence. Calcein blue was used to determine magnesium in blood serum [27]. The strong metal-ligand complex formed perturbed the existing equilibria and therefore, this method was more suitably adapted to the determination of total magnesium instead of the ionic magnesium.

There are complications that are involved with the use of these fluorescent dyes. Cross-sensitivity for other ions is a problem and therefore masking agents are required [24,27]. Also, the dyes that are designed to be specific for magnesium ions tend to have high binding affinities [7]. These problems defeat the purpose of speciation studies. A measurement method must not perturb the series of existing complex equilibria if an accurate determination of the uncomplexed Mg^{2+} ions is desired.

1.5 ^{31}P NMR

Nuclear magnetic resonance (NMR) techniques have been used to evaluate the concentration of uncomplexed magnesium ions in the presence of magnesium-ligand complexes. For intracellular determinations, the level of uncomplexed Mg^{2+} ions can be estimated by the analysis of the ^{31}P NMR signals for adenosine triphosphate (ATP). A mathematical relation is used to equate the chemical shift data with the concentration of uncomplexed Mg^{2+} ions. However, there are factors which affect the validity of this approach. The equations are biased by ATP binding to hemoglobin [28]; constants are affected by both the pH and the ionic strength of the test sample [29-32]; additional complexation by competing ligands is not accounted for [29,32,33]; sensitivities of chemical shift data are insufficient, and; a knowledge of the total concentration of ATP is required [29-31]. A similar method uses a ^{31}P NMR indicator which complexes Mg^{2+} ions [34]. Since the indicator has a high affinity for Mg^{2+} ions, the existing equilibria are necessarily perturbed which leads to inaccurate determinations.

1.6 OTHER METHODS

Other techniques have also been used to analyze systems for their magnesium ion concentration. Variations of such methods as flow injection analysis (FIA), complexation and column chromatography have been used for this purpose.

Yamane and Goto have reported a FIA method for the simultaneous determination of calcium and magnesium ions [35]. However, this involves sample dilution, the addition of a masking agent and the formation of a complex with a spectrophotometric reagent. Each of these perturb the original equilibria in a system and the result is therefore not a measure of the original conditions.

A similar study by Marcos et al [36] avoided the problem of interfering ions by utilizing the different degrees of complexation of the metal ion at varying pH's. Again, the addition of a strong complexation agent causes changes in the original equilibria of the system. Also, pH changes affect the conditional formation constants and therefore, the distribution of the species is changed.

Similar methods by Lima et al [37] (dialysis), Swaile et al [38] (capillary zone electrophoresis) and Girotti et al [5] (continuous flow) involved the addition of a complexing agent and subsequent analysis. These methods could be extended to be useful as inexpensive alternatives to atomic absorption spectrophotometry in determining total magnesium in a sample, however, they are not useful for speciation studies.

Two other approaches for the determination of Mg^{2+} ions involve column chromatography. Iwachido et al [39] showed that Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} ions in a solution could be separated using a silica gel column. However, it was

necessary that the pH and ionic strength be fixed. Under these conditions, the retention modifier (mobile phase containing a complex ligand), in this case lithium oxalate, would have a defined distribution. In solutions with various equilibria, the addition of a retention modifier under specific conditions would cause shifts in the equilibria and therefore, the results obtained would be inaccurate.

CHAPTER TWO

EXPERIMENTAL

2.1 CHEMICALS, SOLVENTS AND GASES

Chloromethyl ethyl ether (96%); chlorosulfonic acid (99%); 25% glutaraldehyde solution; 2-methyl pyridine 1-oxide (98%); 5-nitro-1,10-phenanthroline (98%); 5% palladium on carbon; phosphorous trichloride (99.99%); potassium cyanide (97%); potassium permanganate (99%); stannic chloride (99%), and; stannous chloride dihydrate (98%) were all used as received from Aldrich.

Fine copper metal from Allied Chemical was used to prepare stock Cu^{2+} solutions.

Acetone (ACS); ammonium hydroxide (concentrated, 25% NH_3); dimethylformamide (ACS); dimethyl sulfoxide (ACS); 95% ethanol; glacial acetic acid (ACS); methanol (ACS); nitric acid (concentrated, ACS); sodium hydroxide pellets (98%); sodium perchlorate (99%); sulfuric acid (ACS), and; toluene (ACS) were all used as received from BDH.

Ammonium bromide (ACS); chloroform (ACS); dichloromethane (ACS); disodium ethylenediaminetetraacetate (ACS); magnesium metal (40-60 mesh); oxalic acid (ACS); sodium acetate (ACS); sodium bicarbonate (ACS); sodium perchlorate (purified), and; sodium phosphate monobasic (ACS) were all used as received from Fisher.

Purified acetylene and prepurified nitrogen gas were obtained from Linde.

CPG-aminopropyl and CPG-8-hydroxyquinoline (Pierce) had a particle size of 125-177 μm and pore diameter of 500 Å.

N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) (HEPES) and picolinic acid (99%) were used as received from Sigma.

2.2 PREPARATION OF METAL SOLUTIONS

A NANOpure™ system (Barnstead, model# D4751) was used throughout to purify the water used for sample preparation.

All glassware used was soaked in diluted HNO_3 and rinsed with NANOpure™ water prior to being used.

Metal stock solutions (i.e. Cu^{2+} and Mg^{2+}) were made by dissolving the required amount of metal in either 1% or 10% HNO_3.

Solutions were filtered with either a 40-60 μm Coarse Fritted Disc filter funnel (Fisher, #10-358M) or a 0.45 μm Nylon 66 membrane (Alltech, #2024).

2.3 SYNTHESIS OF IMMOBILIZED LIGANDS

The preparation of the solid polymer supports and the immobilization of the following ligands, with the exception of oxine, were done in house by Dr. NaiHong Li.

2.3.1 CPG-OXINE

As mentioned in the CHEMICALS section, this is a commercial product and its structure is shown in Figure 2.1.

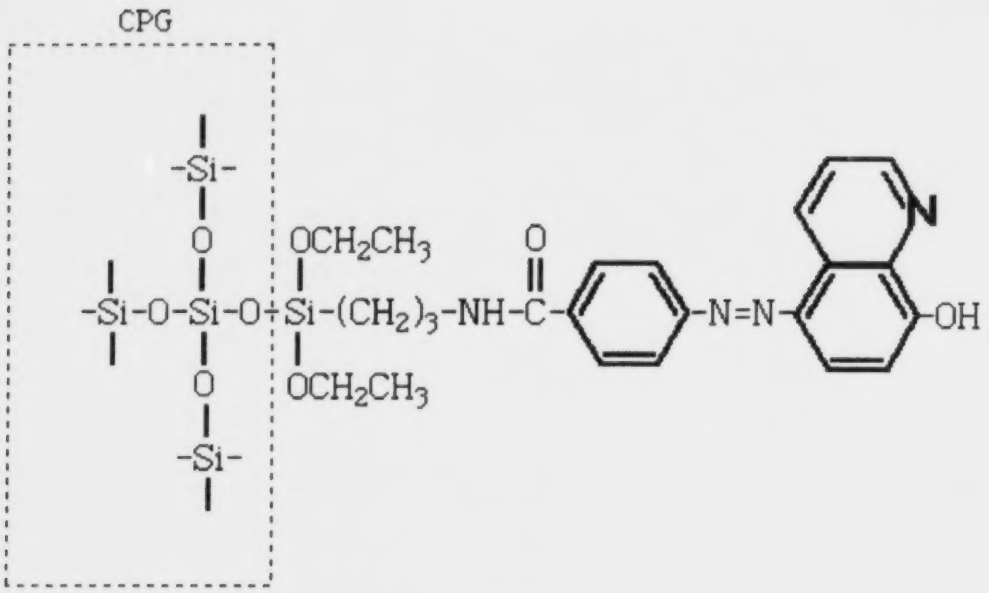


Figure 2.1. The structure of immobilized CPG-Oxine.

2.3.2 CPG-PHENANTHROLINE

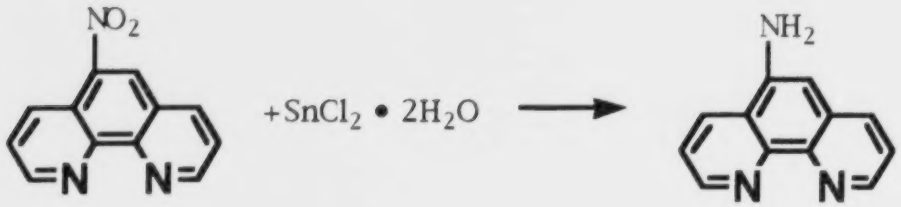
The preparation scheme is shown in Figure 2.2.

2.3.2.1 PREPARATION OF 5-AMINO-1,10-PHENANTHROLINE: To a solution of 1.299 g of stannous chloride dihydrate in 50 mL of methanol, 0.433 g of 5-nitro-1,10-phenanthroline was added and the mixture was refluxed for 4 hours and then it was allowed to stand at room temperature for 6 hours at a pH=1. After removal of most of the methanol, the mixture was made alkaline (pH=12) with a 10% sodium hydroxide solution. The precipitate was removed by filtration and then dried. On crystallization from ethanol, 0.4 g of product was obtained having a melting point of 250-252 °C (literature : 259-260 °C) [40].

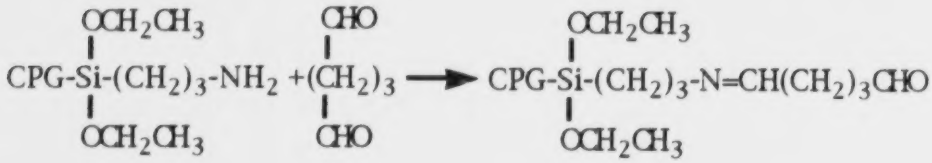
2.3.2.2 PREPARATION OF CPG/ALDEHYDE DERIVATIVE: The reaction is carried out at room temperature. To 1.4145 g of CPG-aminopropyl, 80 mL of a 2.5% solution of glutaraldehyde (25% glutaraldehyde solution diluted in 0.05 M sodium phosphate solution at pH=7) was added. The reaction mixture was placed in a three-necked round bottom flask and attached to an air aspirator to remove air and gas bubbles from within the particles. The reaction continued attached to the aspirator for 2 hours. The product was removed by filtration on a Büchner funnel and then washed with distilled water and methanol.

2.3.2.3 IMMOBILIZATION OF THE LIGAND ONTO THE SUPPORT: A 1.0147 g (wet weight) portion of the CPG/Aldehyde derivative, prepared in 2.3.2.2, was reacted with 0.1190 g of 5-amino-1,10-phenanthroline, prepared in 2.2.2.1, in 50 mL of a 0.05 M sodium phosphate solution at pH=7. The reaction

PREPARATION OF 5-AMINO-1,10-PHENANTHROLINE



PREPARATION OF CPG/ALDEHYDE DERIVATIVE



IMMOBILIZATION OF LIGAND ONTO SUPPORT

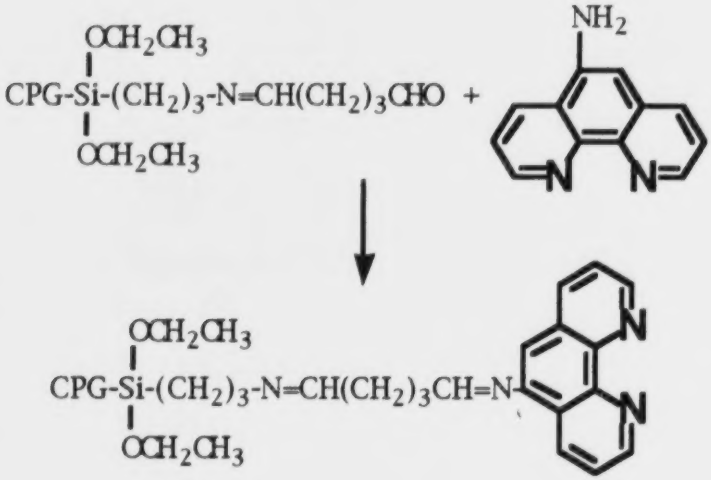


Figure 2.2. A schematic of the preparation of CPG-Phenanthroline.

was carried out under gentle stirring at room temperature overnight. The product was filtered and washed with distilled water. The amount of product (CPG-Phenanthroline) obtained was 0.5752 g [41].

2.3.3 P-PHENANTHROLINE

The preparation scheme is shown in Figure 2.3.

2.3.3.1 MODIFICATION OF POLYMER BEADS: To 1.5 g of porous poly(hydroxyethyl methacrylate/divinylbenzene) (pore size ≈ 400-1000Å) [42] beads in 60 mL of chloroform, 50 mL of chlorosulfonic acid was added. The reaction was continued at room temperature for 1 day. After filtration and washing with chloroform, dichloromethane, methanol, water and then finally, methanol, the product was dried in an oven at 60°C overnight [43].

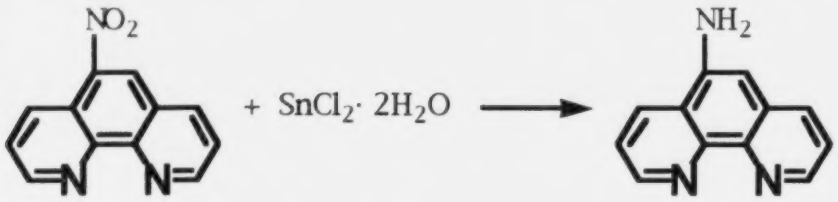
2.3.3.2 IMMOBILIZATION OF THE LIGAND ONTO THE SUPPORT: A 0.5 g sample of the modified polymer beads prepared in 2.3.3.1 were reacted with 0.1 g of 5-amino-1,10-phenanthroline, prepared in 2.3.2.1, in 5 mL of toluene. The reaction was shaken at room temperature for 2 days. The product was filtered and then washed with chloroform, acetone, methanol, water and methanol and dried in an oven at 60°C for 18 hours [44].

2.3.4 P-PHENYLGLYCINE

The preparation scheme is shown in Figure 2.4.

2.3.4.1 MODIFICATION OF POLYMER BEADS: A 3.8 g sample of porous poly(styrene-divinylbenzene) [42] beads were suspended in a solution of 50 mL of chloroform and 10 mL of chloromethyl ethyl ether. 2 mL of stannic chloride was added and the mixture was kept at room temperature for 2 days.

PREPARATION OF 5-AMINO-1,10-PHENANTHROLINE



THE MODIFICATION OF POLYMER BEADS

IMMOBILIZATION OF LIGAND ONTO SUPPORT

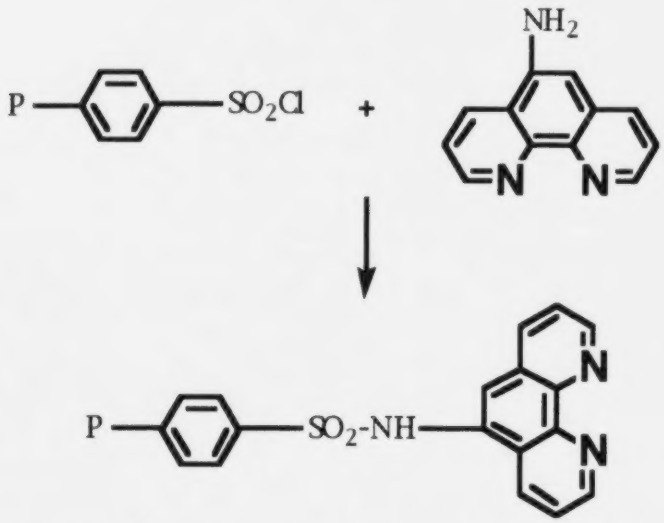
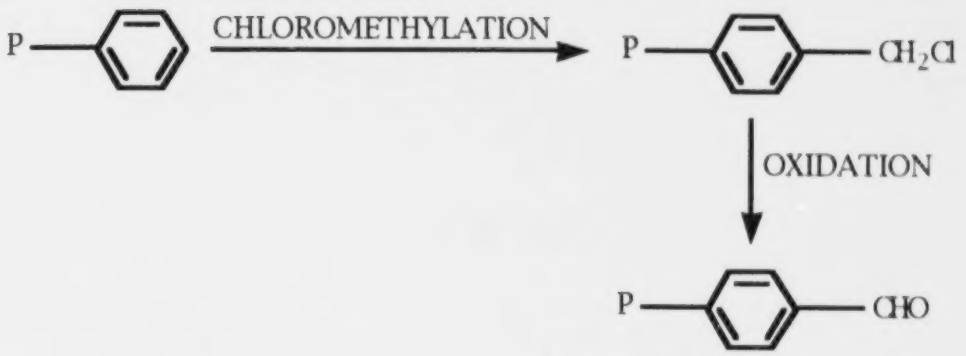


Figure 2.3. A schematic of the preparation of P-Phenanthroline.

MODIFICATION OF POLYMER BEADS



IMMOBILIZATION OF LIGAND ONTO SUPPORT

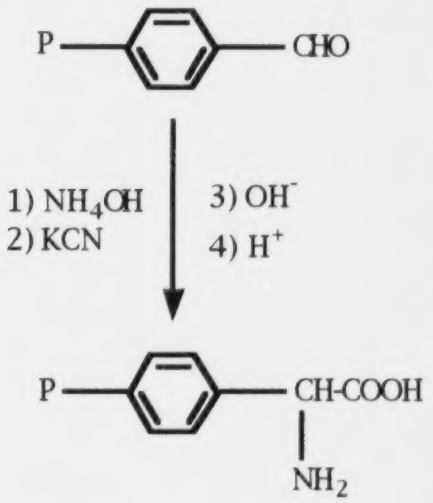


Figure 2.4. A schematic of the preparation of P-Phenylglycine.

The beads were washed completely and then the product was washed with methanol and dried at 60°C for 24 hours.

A 2.426 g sample of this product was reacted with 50 mL of dimethyl sulfoxide containing 1 g of sodium bicarbonate at 150°C for 6 hours and at room temperature for 18 hours. The product was washed with acetone, methanol, water, methanol, acetone and finally with methanol. The polymer beads were then dried at 60°C for 24 hours.

2.3.4.2 IMMOBILIZATION OF THE LIGAND ONTO THE SUPPORT: To a suspension of 1.0 g of the modified polymer, prepared in 2.3.4.1, in 25 mL of methanol, was added 2.7 g of ammonium bromide, 2.55 g of potassium cyanide, 5 mL of concentrated ammonium hydroxide and 15 mL of water. The mixture was heated at 40-50 °C for 5 hours. Then a solution of 8 g of NaOH in 10 mL of methanol and 15 mL of water was added and the mixture was refluxed for 5 hours. The product was isolated by extraction and then neutralized with dilute acid. After filtration and washing with methanol, acetone, dichloromethane, methanol, water and finally methanol, the product was dried at 60°C for 24 hours [45].

2.3.5 P-PICOLINIC ACID

The preparation scheme is shown in Figure 2.5.

2.3.5.1 PREPARATION OF PICOLINIC ACID: To a mixture of nitric acid and sulfuric acid, 10 g of 2-methylpyridine 1-oxide was added slowly, with cooling. The solution was heated on a water bath for 12 hours and then cooled [46].

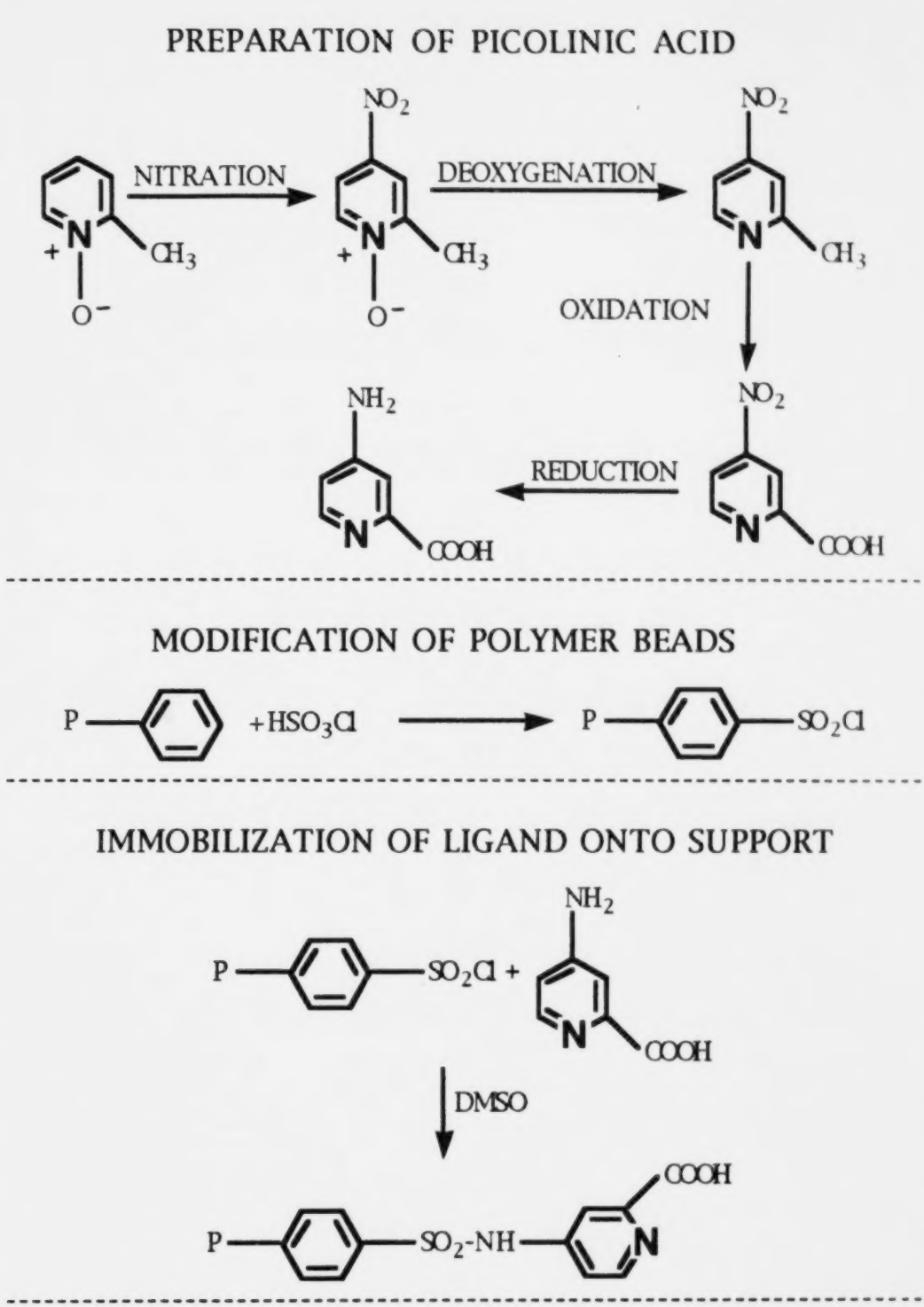


Figure 2.5. A schematic of the preparation of P-Picolinic acid.

The product was mixed with 50 mL of chloroform and 5 g of phosphorous trichloride was then added portionwise. The mixture was warmed at 50°C for 10 minutes and then cooled [47].

This product was then mixed with aqueous potassium permanganate and maintained at a temperature of 75°C for 10 hours [48].

To this product was added 5% palladium on carbon and 25 mL of iron in hydrochloric acid. This mixture was then heated followed by cooling. The catalyst was removed and the product isolated was 4-amino-2-carboxylic acid [49].

2.3.5.2 MODIFICATION OF POLYMER BEADS: A 1.5 g sample of porous poly(styrene/divinylbenzene) [42] beads were placed in 60 mL of chloroform and 50 mL of chlorosulfonic acid was then added. The reaction was continued at room temperature for 1 day. After filtration and washing with chloroform, dichloromethane, methanol, water and then finally, methanol, the product was dried in an oven at 60°C overnight.

2.3.5.3 IMMOBILIZATION OF THE LIGAND ONTO THE SUPPORT: A 0.64 g sample of the modified polymer beads prepared in 2.3.5.2 were reacted with 0.2 g of picolinic acid, prepared in 2.3.5.1, in 10 mL of dimethylformamide solution. The reaction was shaken at room temperature for 2 days. The product was filtered and then washed with chloroform, acetone, methanol, water and methanol and dried in an oven at 60°C for 18 hours.

2.4 EQUIPMENT

pH measurements were made with a pair of glass (Fisher, #13-639-3) and calomel (Fisher, #13-620-51) electrodes using an Accumet Model 320 pH Meter (Fisher).

Samples were analyzed using the technique of atomic absorption spectrophotometry (AAS). The AAS (Perkin Elmer 4000) is equipped with a calcium-magnesium Intensitron™ Lamp (#303-6092). The slit width is set at 0.7 nm and the wavelength of primary interest for magnesium is 285.2 nm. The burner control unit (Perkin Elmer, #057-0858) allows the following conditions to be set : Fuel Pressure, 9 pounds per square inch; Fuel Flow, 26 units; Oxidant Pressure, 30 lbs. per sq. in.; Oxidant Flow, 45 units. Attached to the AAS is a Fisher Recordall® Series 5000chart recorder and it was operated at a setting of 0.01 volts full scale. At this setting, the chart recorder was found to yield a linear response over the entire height of the chart paper. This meant that any signals obtained at this setting were usable. The AAS is equipped with a 10 cm burner head (#0040-0289). It should be noted that the burner head can be inserted in three positions. It can be set in the usual 0° position with the full 10 cm length aligned with the incident light beam, or in one of the two angled positions of approximately 30° and 60°. By using an angled burner, the system can, in effect, be de-optimized since the pathlength has decreased ($A=\varepsilon bc$). This procedure can be used to obtain usable signals.

A Perkin Elmer 290B atomic absorption spectrophotometer was also used in conjunction with a similar experimental design for a set of copper studies. This AAS has a copper Intensitron™ Lamp (#303-6081). The P.E. Burner Regulator #303 allows the following conditions to be set : Fuel Pressure, 8 lbs.

per sq. in.; Fuel Flow, 1440units; Air Pressure, 29 lbs. per sq. in.; Air Flow, 140 units. Attached to the AAS is a Shimadzu CR601 Chromatopac Printer.

2.5 COLUMN EQUILIBRATION APPARATUS

The two designs of the apparatus used in the column equilibration studies are shown in Figures 2.6 and 2.7. The method of choice was the elution of the sample directly into the AAS. This setup could only be used when the signals obtained fell within the linear response range of the AAS. As mentioned in section 2.4, the AAS could actually be de-optimized in order to meet this requirement. However, when signals were obtained that exceeded the workable range of the AAS, the apparatus was modified so that the eluted sample was then collected in a vial for an appropriate time. This allowed for the samples to be diluted by the required factor and then subsequently analyzed by aspiration into the AAS.

The experimental setup for the direct analysis of an eluted sample is shown in Figure 2.6 and a similar setup for studies where the eluted sample is collected is shown in Figure 2.7. The stainless steel water bath (constructed in the Machine Shop, University of Alberta) has dimensions of 80x53x28 (LxWxH) centimeters. A heat/stir unit (Haake, model #E52) is used to set the temperature of the bath. The temperature is usually maintained at 25°C ± 1°C.

The pump-flow system in Figure 2.6 has three valves. V1 is a 4-way slider valve (Altex, #201-02), V2 is a micro-sample injection valve, (CHEMINERT®, Laboratory Data Control/Milton Roy, model #4143090) and V3 is a modified column slider valve.

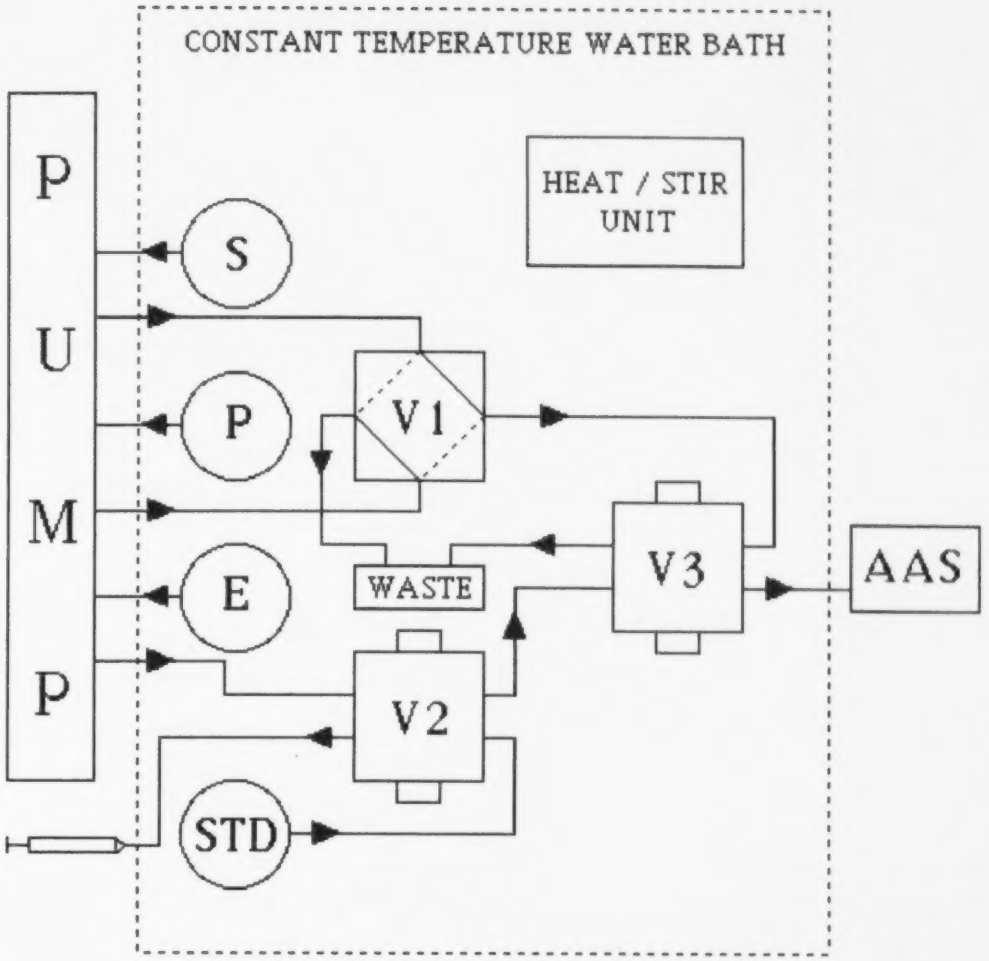


Figure 2.6. Direct elution into the atomic absorption spectrophotometer (AAS). S= sample; P= precondition or water; E= eluent; STD= standard; V1= 4-way valve; V2= standard injection valve; V3= column valve.

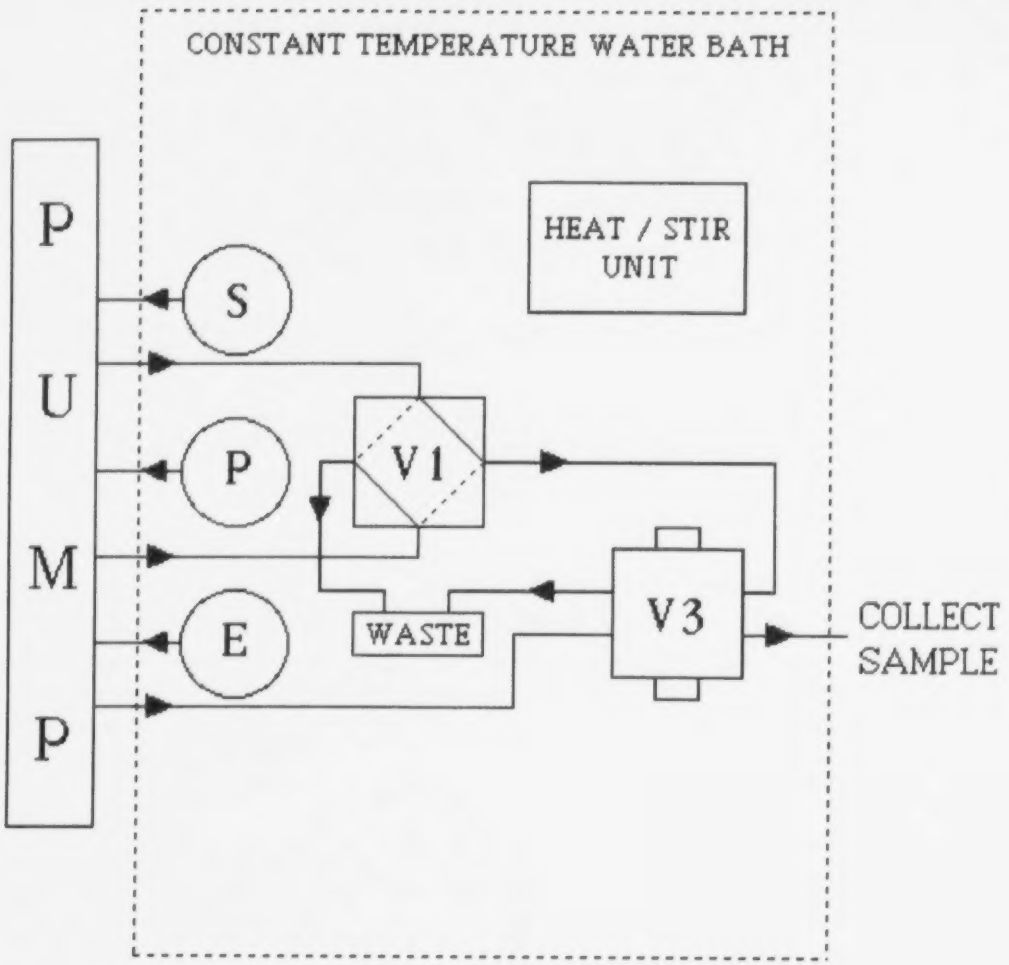


Figure 2.7. Collection of eluted sample. S= sample; P= precondition or water; E= eluent; V1= 4-way valve; V3= column valve.

Sample solution, S, preconditioning solution or water, P, and eluent, E, are pumped (Gilson, Miniplus 2, model #E-82) with Fisher purple-white pump tubes, 0.110" ID, through the system via teflon connecting tubing (Alltech, 1/16" ODx 0.5 mm ID). Typical flow rates are 4.9 mL/min ± 1 mL/min.

The selection between sample solution or water is made using V1. In one position (represented by the solid lines), sample solution flows through V1 and goes to V3, whereas the water flows through V1 and goes to waste. In the other position (represented by the dashed lines), the opposite occurs.

Eluent flows continuously to V3. In the case of Figure 2.6, eluent passes through V2 along its way. The purpose of V2 is to act as a standard injection valve. By retracting the syringe, a 10 μL hole within V1 is filled with the standard solution (STD). This plug of standard solution can then be pushed up into the eluent stream and carried on through the system. This setup is used when V3 is connected directly to the atomic absorption spectrophotometer (AAS) and the standard is used for calibration purposes. In this setup, signals were in the form of peaks and the areas were determined by the on-board digital integrator of the AAS. Note that a standard injection is not required in Figure 2.7.

2.6 ANALYTICAL COLUMN

The analytical column is contained in V3 (Figure 2.8). It makes up the middle hole in the three-holed Kel-F® slider. The two Teflon® face plates are also shown. The three holes of the slider have been modified by the machine shop at the University of Alberta to 2.13 mm in diameter and 6.10 mm in height. The two side holes contain plugs of sintered glass which help to

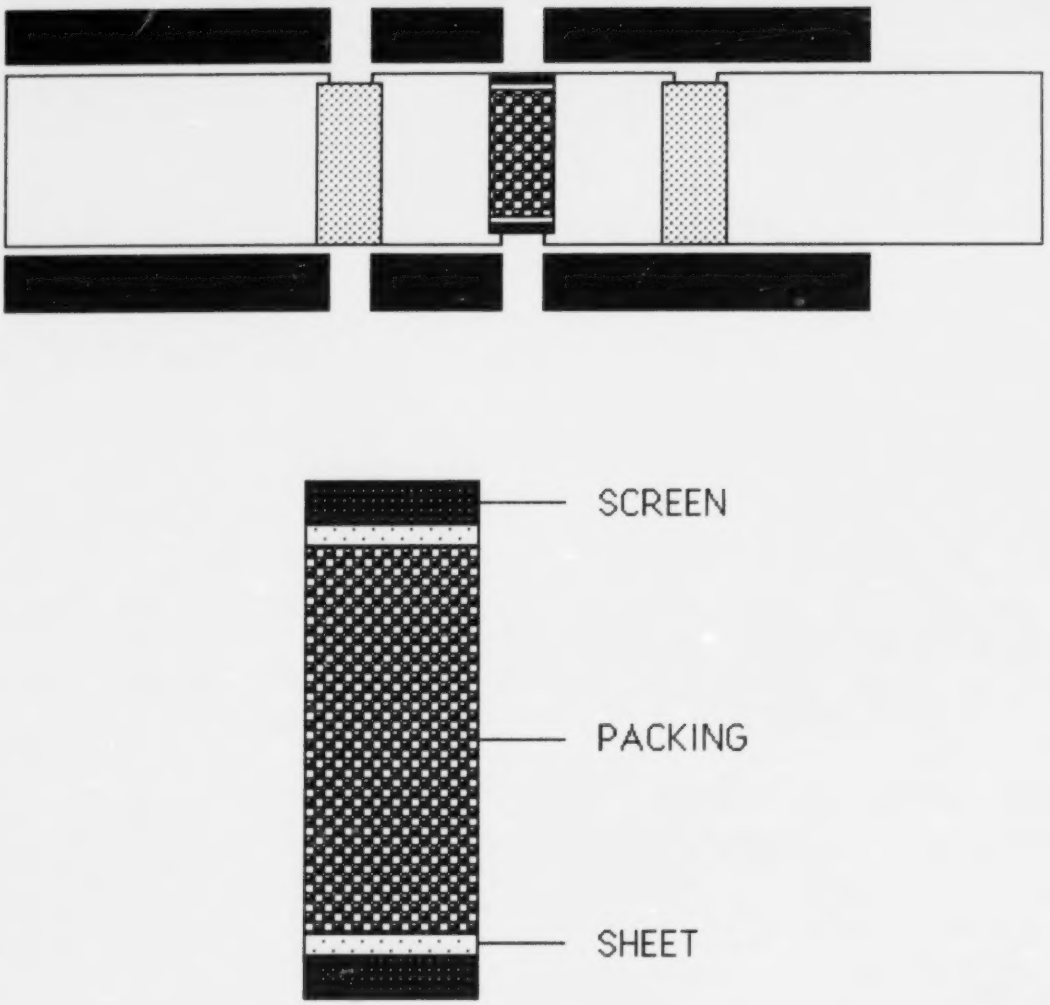


Figure 2.8. Top - A schematic of the slider contained in V3.
Bottom - An enlargement of the column (middle hole).

equalize the flow-through conditions of the three holes. The column consists of a Kel-F® screen which is 2.08 mm in diameter, 0.75 mm high and has 25 holes drilled through it that are 0.33 mm in diameter. A porous Teflon® sheet that is 2.08 mm in diameter and 0.19 mm high with 30-60 μm pores (model #K1064-122D, Zitex, Chemplast Incorporated) is placed between the screen and the packing.

Prior to packing the middle hole, the empty slider and the two screens are first cleaned by putting them into a beaker of 1% HNO_3 and placing this into an Ultrasonic Cleaner (Mettler Electronics Corporation, model ME 4.6) for 10 minutes. These clean pieces are then allowed to air-dry overnight.

The immobilized ligand is first sieved (Canadian Standard Sieve Series) to obtain the desired particle size of 106-150 μm. This material is then washed with methanol, methanol-water and then water. The cleaned material is then allowed to air-dry overnight after which it is dry-packed into the middle hole.

2.7 COLUMN EQUILIBRATION PROCEDURE

In order to perform an experiment using the column equilibration technique, the following procedural steps are usually carried out. First, after the system has had the opportunity to reach the desired temperature as set by the constant temperature bath, the system is rinsed out with the eluent (nitric acid). This is followed by a water rinse of the entire system. The system is then ready to begin the experiment. The sample, eluent and preconditioning solutions are then pumped through their appropriate tubing lines as a final rinse and preparation step. When the direct elution procedure is used (Figure

2.6), practice standard injections are also made to ensure that everything is in the proper working form so that the experiment can be started.

The analytical column is rinsed with eluent, followed by preconditioning solution. Sample solution is then passed through the analytical column (loaded) for the desired amount of time. The eluent is then passed through the analytical column in order to remove the contents contained in the column (elution). For direct elution experiments, the outlet line from V3 is connected directly to the inlet of the AAS and a signal is obtained representing the amount of magnesium that was present in the column. This signal is calibrated by immediately following this signal with magnesium standard injections.

For experiments where sample collection is used, a cleaned vial is placed at the exit of the column valve and the eluted column contents are collected. The appropriate dilutions are performed in order that the magnesium signals fall within the linear calibration range of the AAS. These signals are quantitated by aspirating magnesium standards.

All these steps are carried out by manipulating the positions of the valves (V1,V2,V3) as indicated in Figures 2.6 and 2.7.

These procedural steps are common to most of the investigations carried out in this study. The specifics and any deviations accompany the individual experiments.

2.8 ELUTION CONDITIONS

Before any studies on an immobilized ligand can be done, it is first necessary to examine the elution conditions. It is important to know if all of

the sorbed species is being eluted and also how long this elution takes. Experiments can then be designed accordingly.

The immobilized ligands examined were attached to either CPG or P solid supports. Therefore, it was necessary to investigate the elution conditions for both materials. The general experimental conditions are described below and the specifics are included with the data tables that follow.

The column (V3, Figure 2.6 and 2.7) is first rinsed with water. Then V1 is switched and a sample solution containing a given magnesium concentration is passed through the column for a specified amount of time. V3 is then switched and the column is moved into the eluent stream and the eluted sample passes into the AAS. The signal is followed on the chart paper. As mentioned in section 2.5, the flow rate was 4.9 mL/min. The amount of time for the peak to return to the baseline is the time for complete elution. This procedure is followed using different eluent concentrations. In doing such a study, the effect of eluent concentration on the time for complete elution is determined.

First, the data (Table 2.1) for the CPG-Oxine study indicates that the time for complete elution decreases to a value of 30 seconds with increasing concentration to about 1% HNO_3. Above that it no longer changes. Therefore, 30 seconds is sufficient time to allow for all of the sorbed metal to be eluted from the column. A 1% HNO_3 eluent concentration was also used in the elution studies involving CPG-Phenanthroline. Under these conditions, a sample required 30 seconds for complete elution. With this being the case, experiments for these immobilized ligands were designed knowing that either the peak integration time in the direct elution setup or the collection time in the sample collect setup would have to begin just prior to elution and continue for 30 seconds.

ELUENT CONCENTRATION	ELUTION TIME (MINUTES : SECONDS)
0.5 %	0:55
1 %	0:30
2 %	0:30
5 %	0:30

Table 2.1. ELUTION CONDITIONS FOR CPG-OXINE.
This study was performed using CPG-Oxine.

SAMPLE: $1x10^{-5}$ M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer (pH=7.00). PROCEDURE: Water rinse, 2 min; Load sample, 2 min; Elute with X% HNO_3; Inject standards.

ELUENT CONCENTRATION	ELUTION TIME (MINUTES : SECONDS)
1 %	4:15
2 %	1:45
4 %	0:50
6 %	0:30
8 %	0:25
10 %	0:25

Table 2.2. ELUTION CONDITIONS FOR P-PHENANTHROLINE.
This study was performed using P-Phenanthroline.

SAMPLE: $2.1x10^{-4}$ M Mg^{2+} in X% HNO_3. PROCEDURE: Water rinse, 2 min; Load sample, 4 minutes; Elute with the corresponding X% HNO_3.

IMMOBILIZED LIGAND	ELUENT CONCENTRATION	ELUTION TIME (MINUTES : SECONDS)
CPG-OXINE	1 %	0:30
CPG-PHENANTHROLINE	1 %	0:30
P-PHENANTHROLINE	10 %	0:30
P-PHENYLGLYCINE	10 %	0:30
P-PICOLINIC ACID	10 %	0:30

Table 2.3. Summary of elution conditions for the immobilized ligands investigated in this study.

The data for the P-Phenanthroline study (Table 2.2) shows that a 1% HNO_3 solution does not elute the sample as effectively as the 10% HNO_3 solution. From the data, it can be seen that the time for elution decreases with the increasing eluent concentration and then levels off. Therefore, 10% HNO_3 was chosen as the eluent concentration. The peaks are eluted in approximately 30 seconds. The same eluent (10% HNO_3) was used for the P-Phenylglycine and P-Picolinic acid systems and the times for complete elution were also found to be 30 seconds.

The results of the elution conditions obtained for the five immobilized ligands studied are summarized in Table 2.3

2.9 CAPACITY STUDY

This study involves determining the maximum number of moles of metal that can be sorbed per gram of the immobilized ligand. A knowledge of the capacity of the immobilized ligand is useful since the distribution coefficient is generally linear over only a small percentage of the capacity.

One way of determining the capacity is by elemental analysis. These measurements were performed at the Microanalytical Laboratory at the University of Alberta. From the percent compositions, the capacity can be calculated mathematically.

Another method to determine the capacity uses the column equilibration technique to measure the sorption isotherm of a strongly complexed metal ion, such as Cu^{2+}. The idea is based on a batch method [50] and is modified to the flow-through column equilibration technique. By passing a solution of sufficiently high metal ion concentration through the column

until equilibrium is reached, all the sites available for sorption will be occupied. The signal obtained upon elution can be quantitated and the capacity calculated.

To do this study, it is necessary to optimize the concentration of the metal ion solution, the loading time and the washing time. As an example, the experimental capacity determination of CPG-Phenanthroline will be examined. Since the method is based on literature work, the metal used is copper. For these studies, the PE 290B atomic absorption spectrophotometer is used.

2.9.1 WASHING CONDITIONS IN CAPACITY STUDY

The purpose of this study is to determine the amount of time necessary to rinse out the unbound copper from the column. N_2 sparged water is used in order to avoid a slightly acidic water source (dissolved CO_2) which could potentially lead to the elution of sorbed copper in addition to the washing out of unbound copper. The experimental conditions are given in Figure 2.9.

As shown, a 2 minute wash effectively removes the unbound copper and the absence of a continual decrease indicates that sorbed copper ions are not being eluted off the column by the water. It is also interesting to note the magnitude of the signal with no wash (i.e. wash time = 0 minutes). This signal is due to the combination of the copper that is sorbed by the column material plus the unbound copper ion that is in the entire column volume. As seen, the unbound copper metal represents a large fraction of the total signal (approximately 80%). The factors that effect and give rise to the relative signals of sorbed and unbound metal in the column will be given considerable attention in the sections that follow.

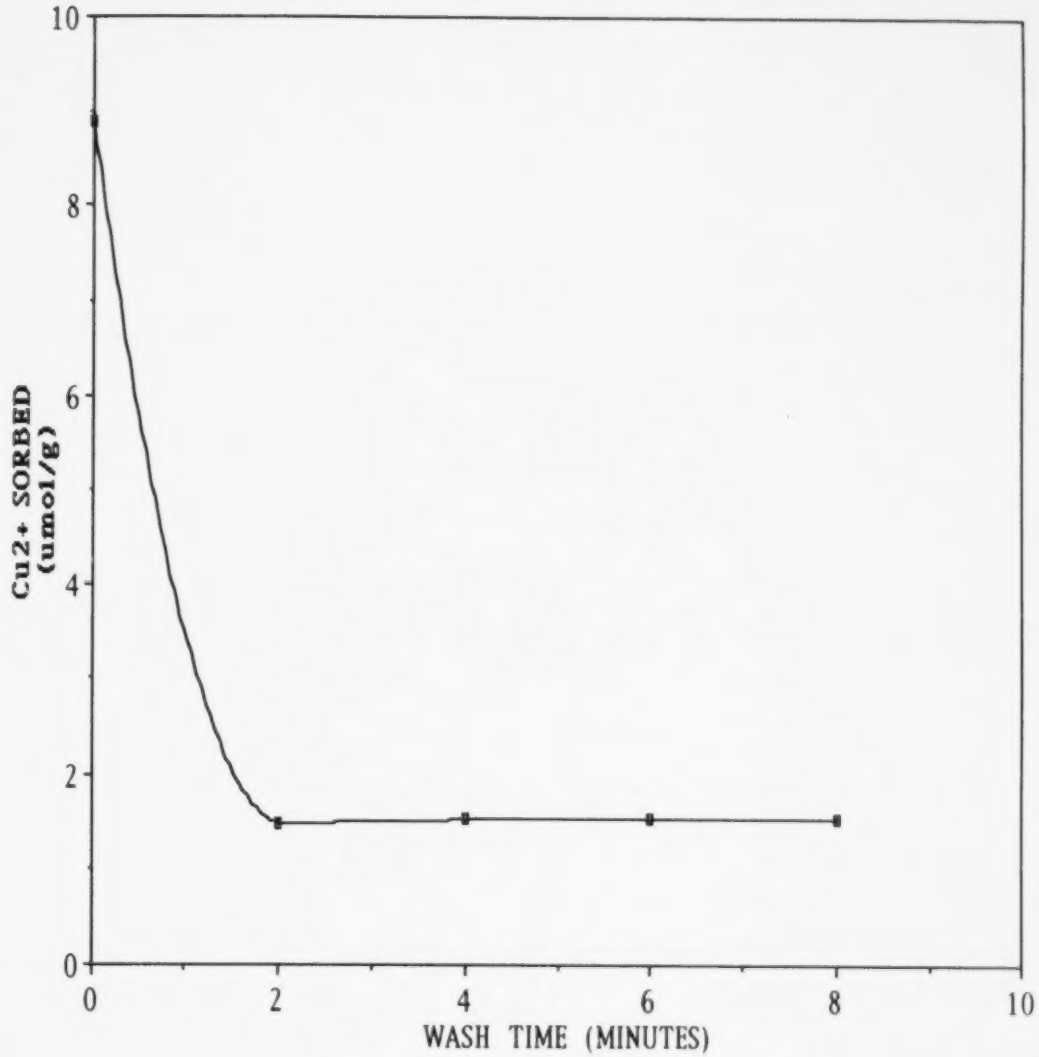


Figure 2.9. WASH STUDY: CPG-PHENANTHROLINE.

SAMPLE: 1.9×10^{-3} M Cu^{2+}; 0.1 M acetate buffer; pH=5.00.
PROCEDURE: Load sample, 2 min; Water wash, X min; Elute; Inject standards.

2.9.2 LOADING CONDITIONS IN CAPACITY STUDY

This study determines the amount of time necessary to pump a copper sample solution through the column in order to reach equilibrium (i.e. sorption levels off). The experimental conditions accompany Figure 2.10 in which it can be seen that a time of at least 2 minutes is required to reach equilibrium.

2.9.3 COPPER SORPTION ISOTHERM IN CAPACITY STUDY

The effect of varying the sample concentration on the amount of copper sorbed is investigated. In Figure 2.11, initially, there is a linear relation - the distribution coefficient, λ_0, is constant. As the sample concentration continues to increase, the sorption reaches a maximum value or plateau at a concentration at about 1.25×10^{-3} M copper. The capacity of CPG-Phenanthroline is then determined from the plateau in the sorption isotherm. The capacity is found to be 1.2 μmol/g.

The capacity of CPG-Oxine was determined in a similar manner. The capacities of P-Phenanthroline, P-Picolinic acid and P-Phenylglycine were determined by elemental analysis. This was done by determining the percentage of nitrogen present in a sample of the immobilized ligand. The capacity was then calculated by finding the number of moles of immobilized ligand present based on the moles of nitrogen present . The capacity data for the five immobilized ligands is shown in Table 2.4.

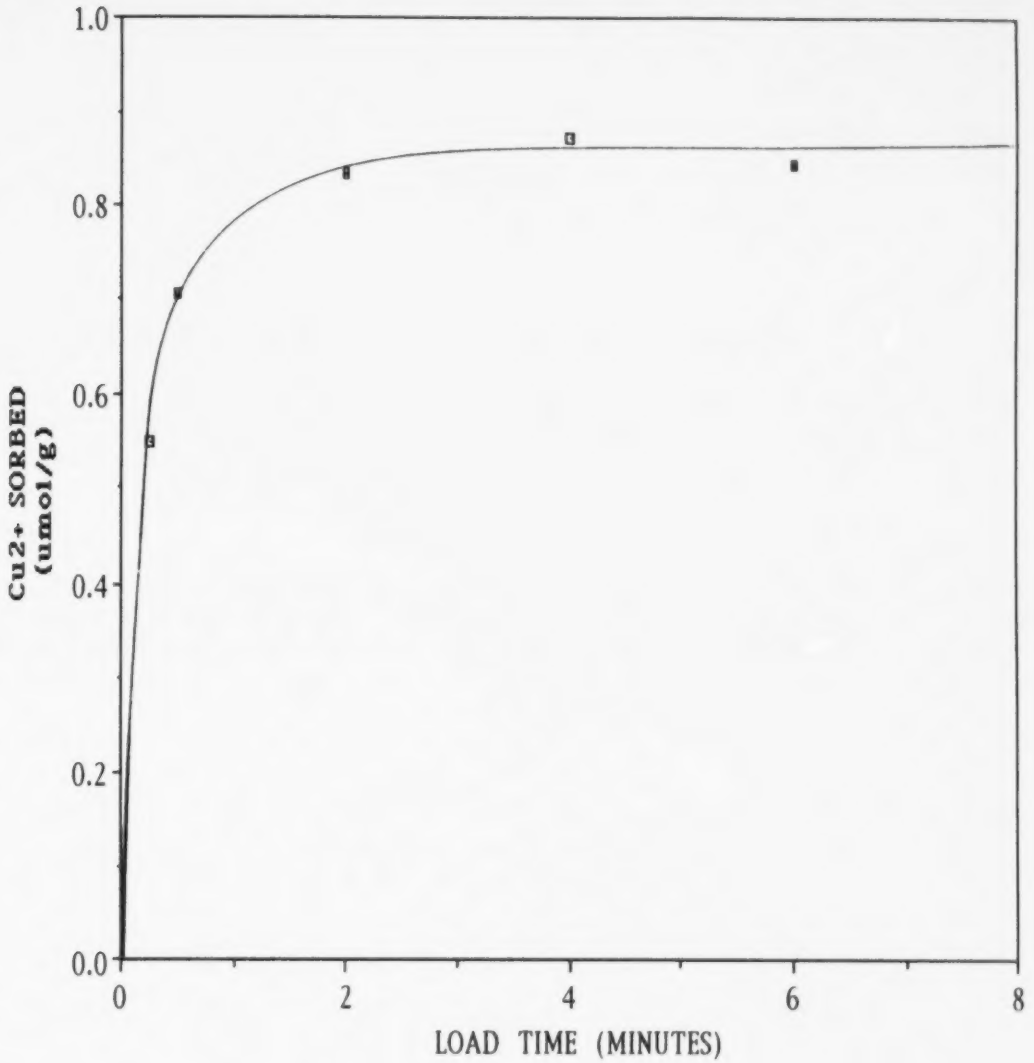


Figure 2.10. LOAD STUDY : CPG-PHENANTHROLINE.

SAMPLE: 1.6×10^{-4} M Cu^{2+}; 0.1 M acetate buffer; pH=5.00.
PROCEDURE: Load sample, X min; Water wash, 8 min; Elute; Inject standards.

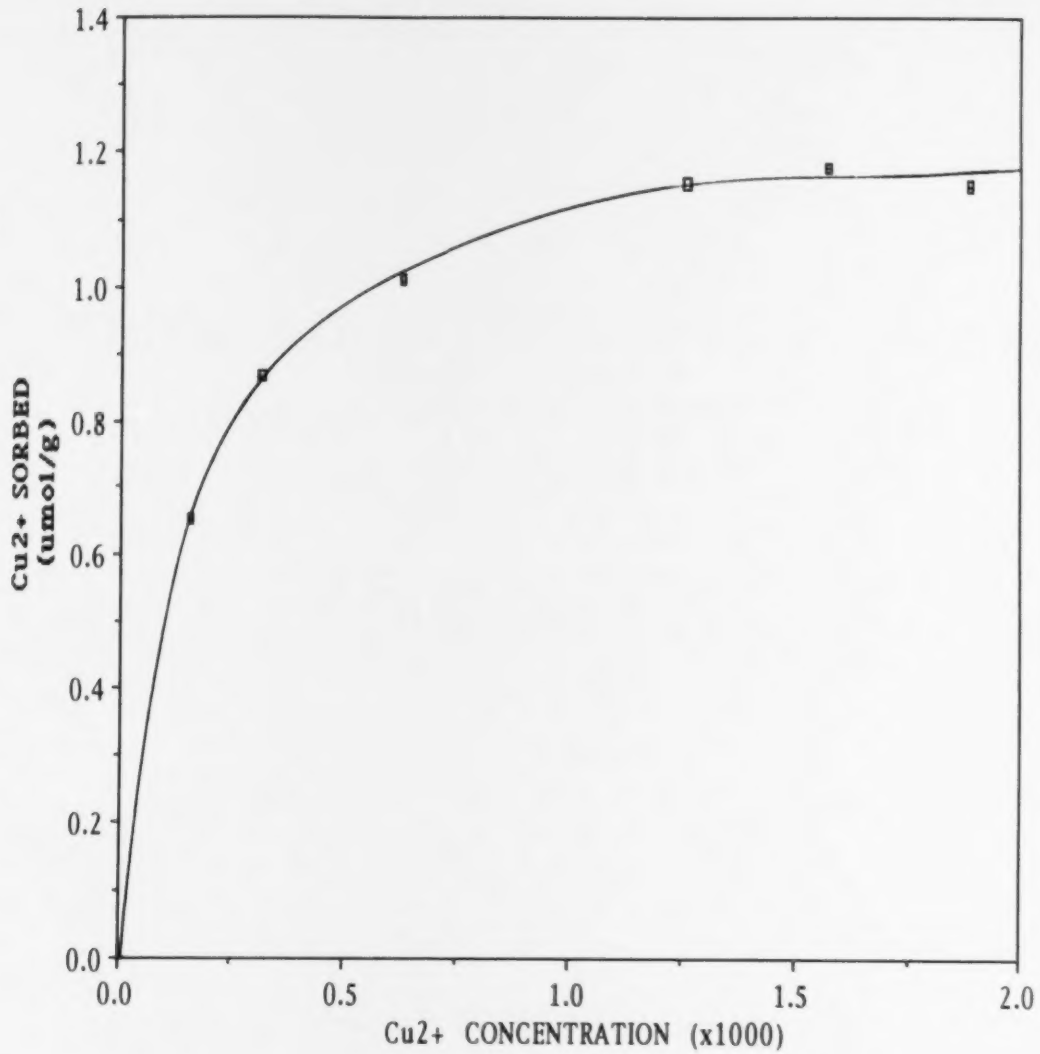


Figure 2.11. COPPER SORPTION ISOTHERM : CPG-PHENANTHROLINE.

SAMPLE: X M Cu^{2+}; 0.1 M acetate buffer; pH=5.00.
PROCEDURE: Load sample, 2 min; Water wash, 8 min; Elute; Inject standards.

LIGAND	CAPACITY	METHOD
CPG-OXINE	9.3 μmol/g	COPPER
CPG-PHENANTHROLINE	1.2 μmol/g	COPPER
P-PHENANTHROLINE	83 μmol/g	ELEMENTAL ANALYSIS
P-PICOLINIC ACID	690 μmol/g	ELEMENTAL ANALYSIS
P-PHENYLGLYCINE	640 μmol/g	ELEMENTAL ANALYSIS

Table 2.4. Capacity values for the immobilized ligands.

CHAPTER THREE

RESULTS AND DISCUSSION

As outlined in Chapter One, there is a need for a reliable method for the determination of the amount of magnesium ion, Mg^{2+}, in samples that also contain other forms of magnesium, such as magnesium bound by organic ligands. Because of the existing simultaneous equilibria in complex solutions, the method for analysis must not disrupt or perturb the test system. Examples of these types of samples would be biological fluids. Blood serum and urine, for example, contain about $1x10^{-3}$ M of total magnesium, of which about 50% is estimated to exist as the uncomplexed or free Mg^{2+} ion [3,4].

The ligands selected for adaptation to the column equilibration technique have been described in Chapter Two. In solution, these ligands are known to form complexes with Mg^{2+}. The formation constants for these ligands with Mg^{2+} are given in Table 3.1 [51]. These solution values are also used to represent the immobilized ligand situation.

The five immobilized ligands are investigated for their Mg^{2+} sorption characteristics. The goal is to find an immobilized ligand that can be used for the determination of Mg^{2+} at concentrations in the millimolar range, since that is the expected range of $[Mg^{2+}]$ in the ultimate target sample solutions of blood and urine. The immobilized ligands that exhibit analytically useful calibration responses in the millimolar range for Mg^{2+} are then examined for their selectivity for the Mg^{2+} species.

LIGAND	REACTION	LOG K VALUE
OXINE	$H^+ + Ox^- \Leftrightarrow HOx$ $H^+ + HOx \Leftrightarrow H_2Ox^+$ $Mg^{2+} + Ox^- \Leftrightarrow MgOx^+$	9.66 (25°C, 0.1 M) 4.99 (25°C, 0.1 M) 4.31 (25°C, 0.1 M)
PHENANTHROLINE	$H^+ + Ph \Leftrightarrow HPh^+$ $H^+ + HPh^+ \Leftrightarrow H_2Ph^{2+}$ $Mg^{2+} + Ph \Leftrightarrow MgPh^{2+}$	4.93 (25°C, 0.1 M) 1.9 (25°C, 0.1 M) 1.2 (20°C, 0.1 M)
PHENYLGLYCINE	$H^+ + PG \Leftrightarrow HPG^+$ $H^+ + HPG^+ \Leftrightarrow H_2PG^{2+}$ $Mg^{2+} + PG \Leftrightarrow MgPG^{2+}$	9.57 (25°C, 0.1 M) 2.36 (25°C, 0.1 M) 2.22 (25°C, 0.1 M)
PICOLINIC ACID	$H^+ + P^- \Leftrightarrow HP$ $H^+ + HP \Leftrightarrow H_2P^+$ $Mg^{2+} + P^- \Leftrightarrow MgP^+$ $Mg^{2+} + 2P^- \Leftrightarrow MgP_2$	5.39 (25°C, 0 M) 1.01 (25°C, 0 M) 2.58 (25°C, 0 M) 3.95 (25°C, 0 M)

Table 3.1. Formation constants for the immobilized ligands [51].

3.1 Mg^{2+} SORPTION PROPERTIES

As eluded to earlier in the WASH STUDY section of the CAPACITY DETERMINATION, there is a relationship between the amount of metal ion in the column that is sorbed (or bound) to the immobilized ligand and the amount of metal ion that is unbound and exists in the interstitial liquid. An understanding of this relationship and the factors that influence it are necessary in this work. Therefore, a series of immobilized ligands are investigated with the observed results and properties of one influencing the design of the next. By following this method, the new immobilized ligand addresses the deficiencies of the previous immobilized ligand.

In order to be sorbed by the immobilized ligand (L_R), the metal ion in solution (M) must be able to form a complex (ML_R) with the immobilized ligand. The magnitude of this complexation (the formation constant, K_f) is one of the factors which influence the ratio of the amount of metal bound (complexed) to the immobilized ligand to the concentration of the metal ion species in solution.

$$M + (L)_R \Leftrightarrow (ML)_R \qquad K_f = \frac{[ML]_R}{[M]\cdot[L]_R} \tag{3.1}$$

Note that the charges of the ions have been left off for a generalization. Also, the subscript, R, refers to the species in the resin phase. The formation constant affects how much metal ion in solution is sorbed at equilibrium - a higher constant results in a higher ratio of bound to unbound metal ion.

Another important factor in these studies involves the influence of the surface coverage of the immobilized ligand on the solid support. This is referred to as the capacity. It is apparent that if there are more available

complexing sites (i.e. a higher capacity) that the value for the amount of metal ion sorbed at equilibrium would be larger.

The inter-relationship between the formation constant and the capacity will be examined in this section. The effect that these factors have on the linear sample capacity (i.e. the range over which the distribution coefficient is constant) can then be used to select or design an improved immobilized ligand with the appropriate conditions.

There are three general types of experiments that have been carried out and they are outlined in the following section. The specifics of each immobilized ligand are then given with a discussion of the progression from one immobilized ligand to the next included.

The investigation of the sorption characteristics of the different immobilized ligands involved the selection of an appropriate sample solution. First of all, the concentration range for Mg^{2+} ion was decided on based on the estimated values in biological fluids [3,4] and on previous results obtained within the research group[11]. The concentration range used was between 10^{-5} and 10^{-3} M. Other typical parameters reported for biological fluids include an ionic strength range of 0.1 to 0.3 M [4] and a pH range of 5 to 8. Sodium perchlorate ($NaClO_4$) was used as the ionic strength adjuster since the perchlorate anion does not complex with Mg^{2+} (sodium chloride, NaCl, for example, could not be used since the chloride anions form complexes with Mg^{2+}). The biological buffer HEPES was used since it is a useful buffering agent in the pH 5 to 8 range and also because it complexes negligible amounts of metal ions. Finally, the solutions were filtered prior to use in order to remove any insoluble impurities that could lead to plugging of the analytical column when passed through the column equilibration system.

The **loading curve** is then determined using the prepared sample solution. This investigation determines the amount of time necessary for a sample solution to pass through the column until equilibrium is reached (i.e. no further sorption occurs). Sample solution is passed through the column for a given amount of time, after which the contents of the column are eluted and a Mg^{2+} signal is obtained. This procedure is repeated with various loading times in order to obtain the loading curve.

The signal obtained from the elution of the contents in the column is a combination of the metal which is sorbed on the immobilized ligand and the metal which is in the interstitial solution (or the **hold-up volume**). The sorbed metal is of interest and therefore, the hold-up volume needs to be determined in order that the amount of Mg^{2+} in it can be subtracted out.

One way of doing this is to load an acidified metal solution (a nominal metal concentration made up in the acidic eluent) through the column for the equilibrium loading time. The principle here is that no sorption will occur from the acidified sample. When the contents of the column are eluted, the signal obtained will be that of the Mg^{2+} in the hold-up volume (HUV).

With this knowledge, the values determined in the load study can be corrected.

$$\text{MOLES } Mg^{2+}_{\text{SORBED}} = \text{MOLES } Mg_{\text{TOTAL}} - \text{MOLES } Mg^{2+}_{\text{HUV}} \qquad (3.2)$$

The **sorption isotherm** is a plot of the amount of metal ion sorbed by the resin versus the concentration of the metal ion in solution. From it, the concentration range of metal ion in solution that gives a linear response is obtained. A series of sample solutions of varying Mg^{2+} concentration are passed through the column, one at a time, for the equilibrium loading time and

then the contents of the column are eluted. The magnesium signals obtained are corrected for the hold-up volume contribution and then plotted. The extent of the linear range response is an important sorption characteristic of the immobilized ligands investigated.

3.1.1 CPG-OXINE

3.1.1.1 LOADING CURVE

As seen from the curve in Figure 3.1, there is an initial rise to a pseudo or "overshoot" of equilibrium between 1 and 4 minutes at about 0.72 μmol/g of Mg^{2+}. This is followed by a slow decrease to the actual equilibrium point of 0.63 μmol/g at about 30 minutes. This phenomena was explained in a similar study using calcium [52]. The loading curve for the sorption of Ca^{2+} on CPG-Oxine shows the same characteristics as the Mg^{2+} loading curve. The slow uptake of Na^{+} ions into the hydrated layer of the CPG-Oxine leads to a change in the surface potential of the CPG with the consequent desorption of Ca^{2+} ions. The same rationale is used to explain the observed Mg^{2+} loading curve.

3.1.1.2 ISOTHERM

Examination of Figure 3.2 reveals that there exists a linear relationship up to about 2.5×10^{-5} M Mg^{2+}. Below this region, the distribution coefficient for the ratio of sorbed Mg^{2+} ion to unbound Mg^{2+} ion in solution is constant. After this point, the distribution ratio becomes non-linear. In other words, more than the limited number of complexation sites are being used in the sorption

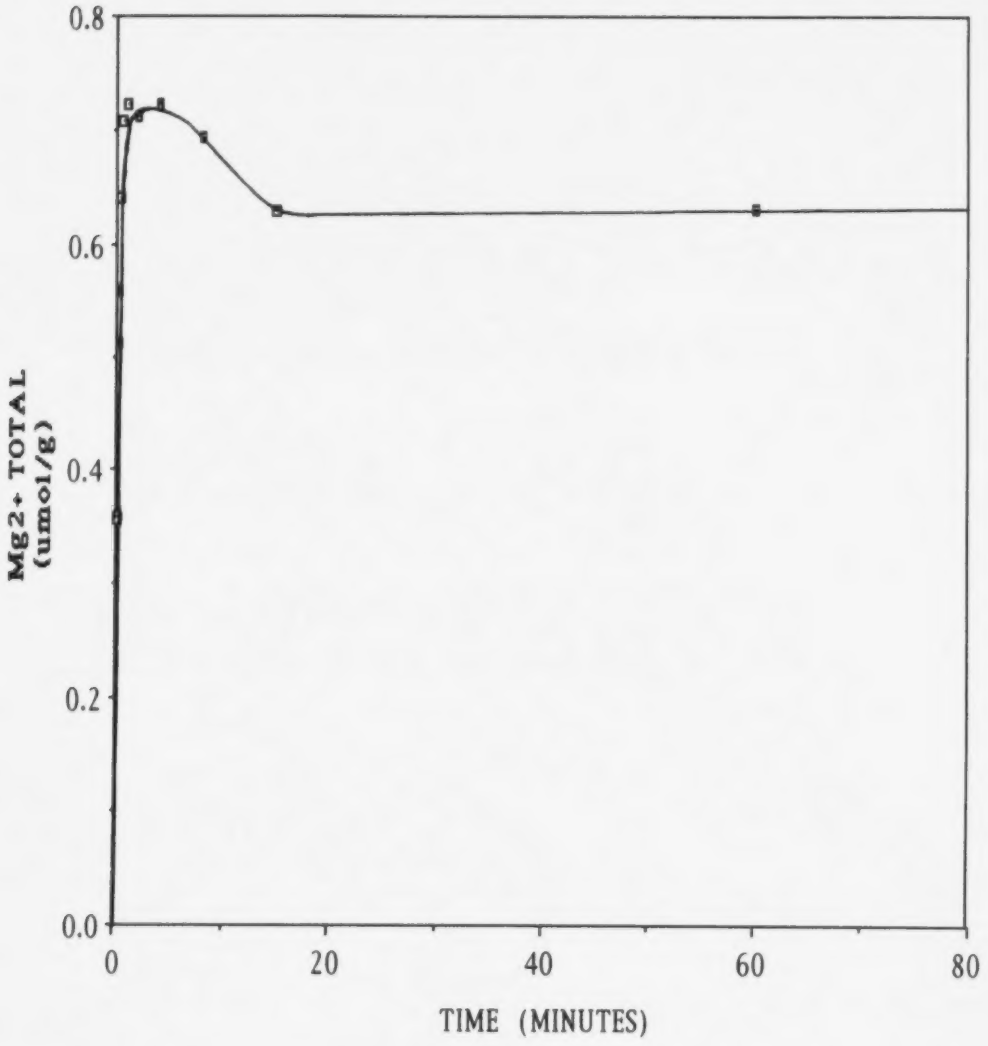


Figure 3.1. LOADING CURVE : CPG-OXINE

SAMPLE: 2.5×10^{-5} M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=7.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Inject standards.

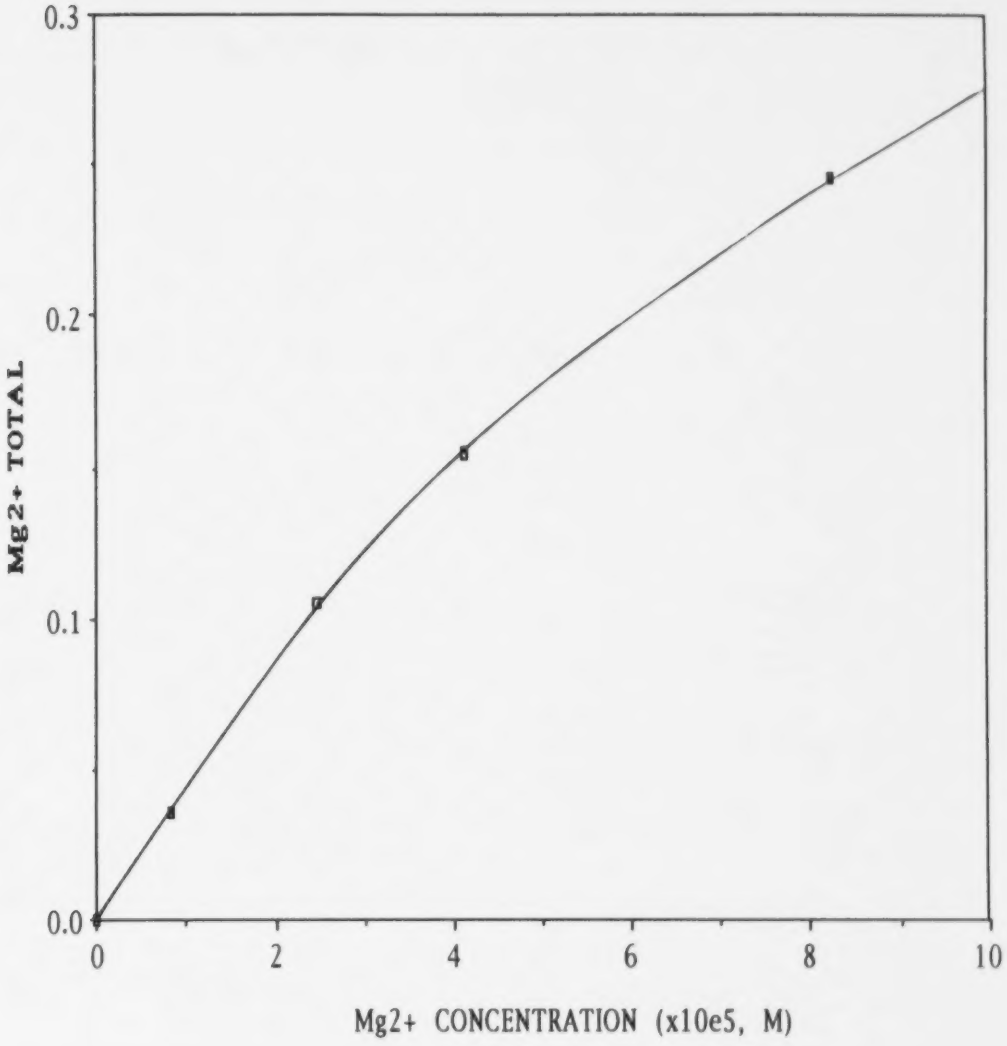


Figure 3.2. ISOTHERM : CPG-OXINE

SAMPLES: X M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=7.00. PROCEDURE: Water rinse, 2 min; Load sample, 2 min; Elute; Inject standards.

of Mg^{2+} ions so that $[L]_R$ has decreased by a significant amount and therefore trace loading conditions are no longer satisfied. The hold-up volume was determined to be 16μL which corresponded to a correction of 0.09 μmol/g. A similar isotherm study for Ca^{2+} with CPG-Oxine has been reported [52].

3.1.1.3 RECOMMENDATIONS

These findings led to the first assessment of the immobilized ligands. The isotherm for CPG-Oxine indicated that the linear sample capacity needed to be extended since the value of $2.5x10^{-5}$ M Mg^{2+} was lower than the desired millimolar level. It was decided that in order to increase the linear range, the sorption of Mg^{2+} needed to be decreased. Based on this, a new immobilized ligand was prepared which had a lower formation constant with Mg^{2+}. Compared to CPG-Oxine, with everything else being equal, this new immobilized ligand would therefore sorb less Mg^{2+} at equilibrium.

3.1.2 CPG-PHENANTHROLINE

3.1.2.1 LOADING CURVE

As seen in Figure 3.3, the load study for Mg^{2+} ion on CPG-Phenanthroline indicates that the "overshoot" of equilibrium is not observed with this immobilized ligand. This can be explained by the relatively slow rise to equilibrium for this system. Since the sorption of Mg^{2+} ions and Na^{+} ions are occurring at more similar rates, the equilibrium value is not exceeded. Also, the curve does not show the levelling off of an expected loading curve. By

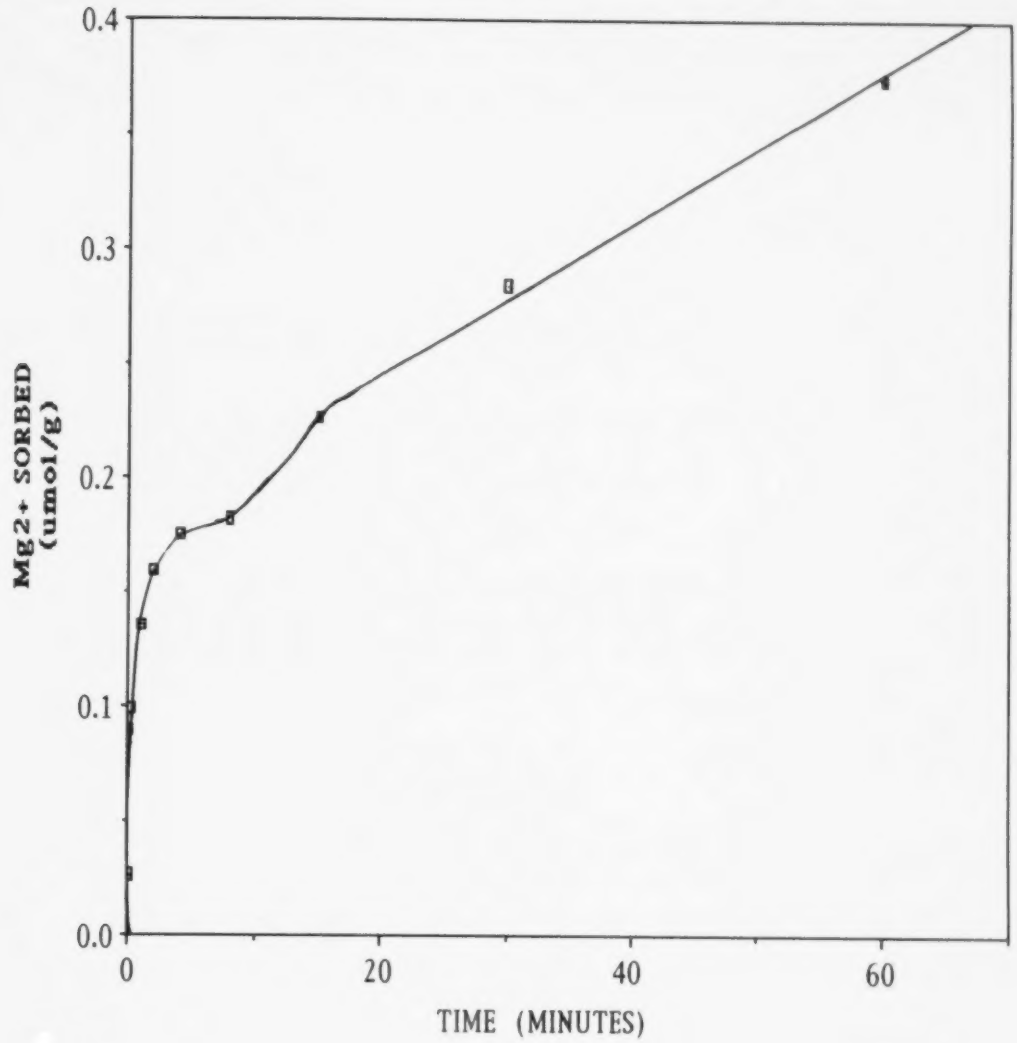


Figure 3.3. LOADING CURVE : CPG-PHENANTHROLINE

SAMPLE: 1.5×10^{-4} M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=7.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Inject standards.

about 8 minutes the curve seems to level off and approach apparent equilibrium. However, at longer times there is a sharp, linear increase in the sorption values. At the time of this investigation, the results at the longer loading times were not investigated. However, as will be seen in section 3.1.4, this peculiarity can be explained.

It is not directly obvious from the load study that relatively less Mg^{2+} sorption is occurring in this system as compared to the CPG-Oxine system since the sample solutions for the load studies are different. However, this will be evident from the isotherm study.

3.1.2.2 ISOTHERM AND HOLD-UP VOLUME

A lower formation constant results in a smaller distribution coefficient. In this system, it is also true that the capacity is lower than for the CPG-Oxine system. From the analysis of Figure 3.4, it is obvious that there is a considerable **blank** or hold-up volume signal in this system. In order to obtain only the signal due to the sorbed Mg^{2+}, the magnitude of the solution concentration of Mg^{2+} contributing to the overall signal must be subtracted out. Generally, when two very similar numbers are subtracted from one another, the resulting small difference is an unreliable value. As a result, the amount Mg^{2+} sorbed is expected to have some scatter or deviation in the values obtained. This is not the case (Figure 3.5). However, this method for obtaining the sorbed values is still considered suspect at best.

This complication aside, the apparent linear range of this system is up to about $3x10^{-4}$ M Mg^{2+}. It is interesting to note the difference in sorption between this system and the CPG-Oxine system. In the CPG-Oxine case, a $2.5x10^{-5}$

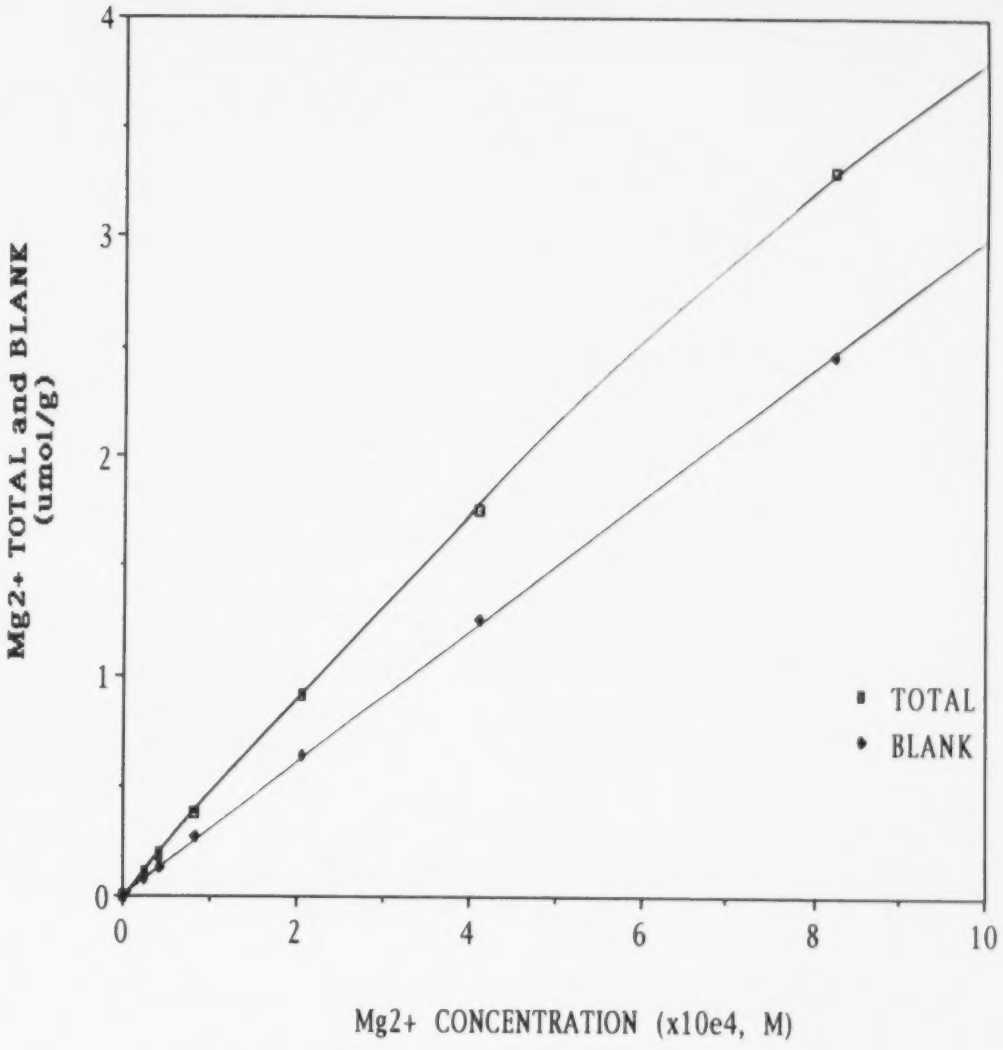


Figure 3.4. ISOTHERM / BLANK : CPG-PHENANTHROLINE

SAMPLES: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=7.00. BLANKS: 1 % HNO_3. BOTH: X M Mg^{2+}. PROCEDURE: Water rinse, 2 min; Load solution, 5 min; Elute; Inject standards.

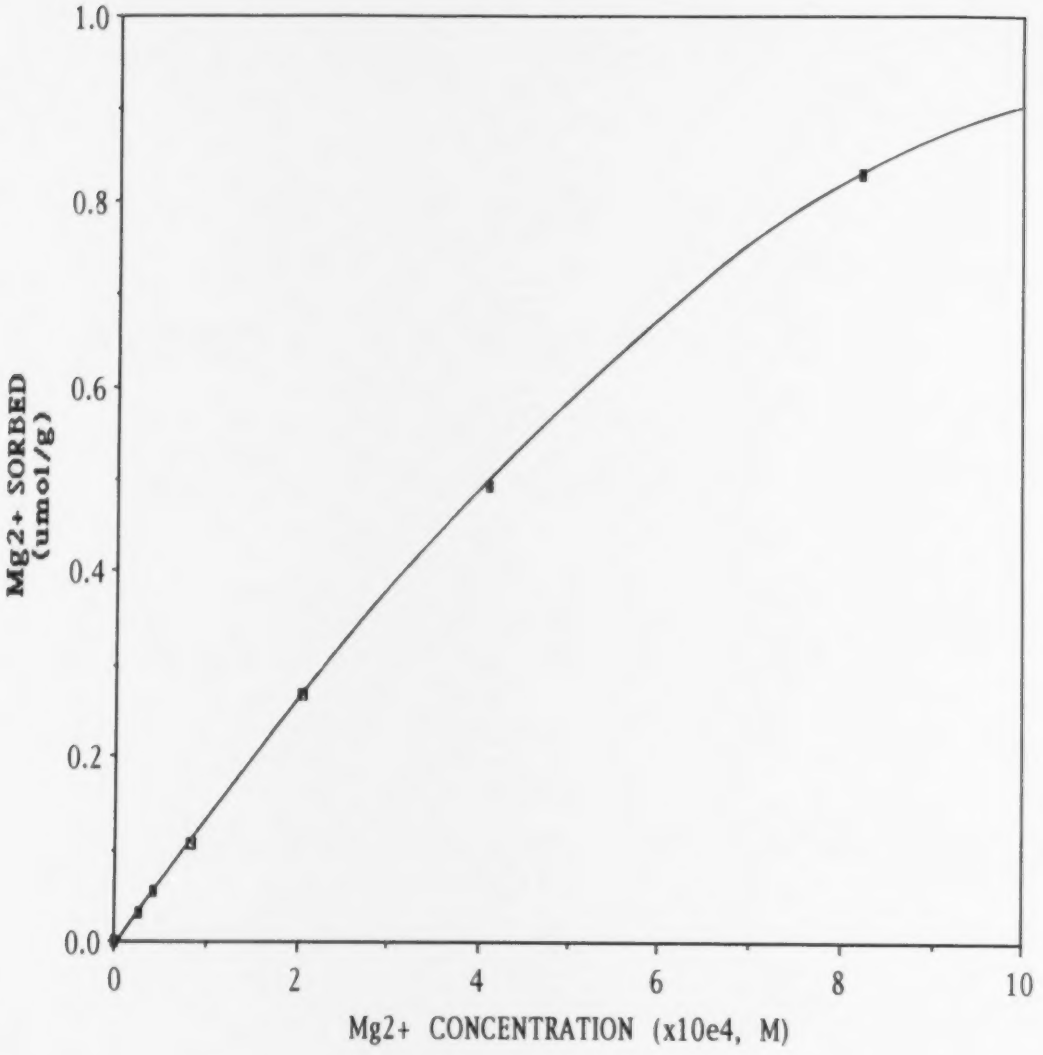


Figure 3.5. ISOTHERM : CPG-PHENANTHROLINE

This curve is obtained by subtracting the blank signal from the sample signal.

M Mg^{2+} solution results in 0.72 μmol/g sorption whereas a similar solution for the CPG-Phenanthroline has only 0.03 μmol/g sorption.

3.1.2.3 RECOMMENDATIONS

The observed linear sample capacity from CPG-Phenanthroline is a substantial improvement over the CPG-Oxine system (1 order of magnitude). However, the presence of such a large blank value is not desirable. It was therefore decided to try increasing the capacity of the immobilized ligand. By doing so, it is expected that the sorbed Mg^{2+} would be increased since there would be more sites available for complexation. This would result in a relatively smaller signal for the hold-up volume and therefore more certainty in the analysis. In order to accommodate the increased capacity, it is necessary to switch from using CPG as the support to a polymer material.

3.1.3 P-PHENANTHROLINE

The polymer support is poly(HEMA/DVB) as indicated in the EXPERIMENTAL section. The HEMA moiety makes the support hydrophilic in nature.

3.1.3.1 LOADING CURVE

Examination of the μmol/g values (Y axis) in Figure 3.6 indicates that the sorption of Mg^{2+} ions has increased with this system as compared to the CPG-Phenanthroline case (Figure 3.5). A similar solution resulted in about 0.03

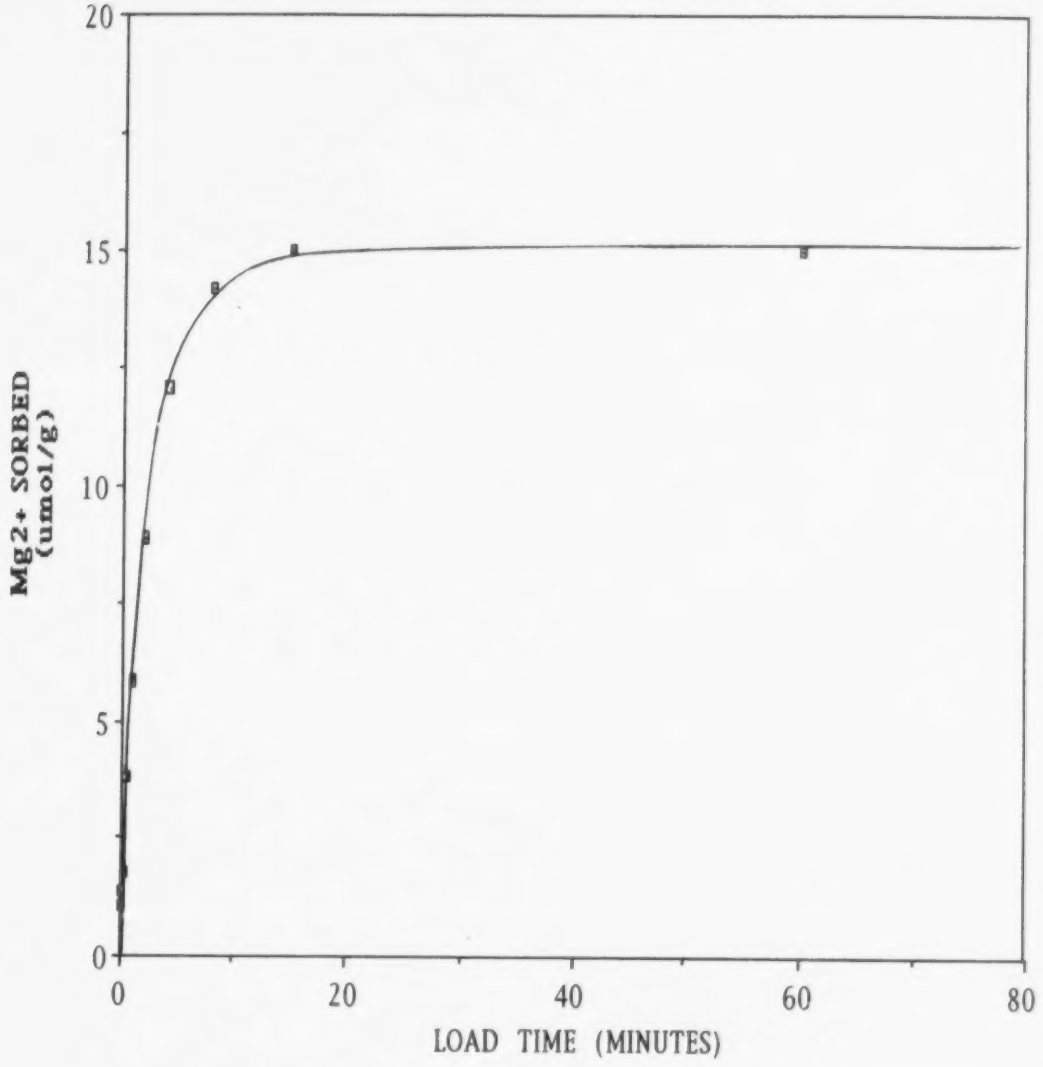


Figure 3.6. LOADING CURVE : P-PHENANTHROLINE

SAMPLE: 2.5×10^{-5} M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=7.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Inject standards.

μmol/g for the CPG-Phenanthroline studies whereas the equilibrium value for the P-Phenathroline is 15 μmol/g. This immobilized ligand requires an equilibrium loading time of 15 minutes. It is evident that the same ligand on different supports has a different loading time. The increased sorption for this system required an increase in the eluent strength to 10% HNO_3 in order for the desorption of Mg^{2+} to occur in the desired amount of time.

3.1.3.2 ISOTHERM

There are a couple of very obvious conclusions that can be made from Figure 3.7. The blank value that was such a problem in the CPG-Phenanthroline study has been eliminated with this system. Analysis of the isotherm values shows that the sample sorption values have increased substantially. The obtained corrected values plotted in Figure 3.8 are reliable since they are the difference between large sample signals and small blank values.

However, on the downside, it is also evident from this system that the corrected values for the μmol/g of sorbed Mg^{2+} are higher than the capacity of the P-Phenanthroline system (83 μmol/g, Table 2.4). This indicates that Mg^{2+} ions are being sorbed onto the backbone of the hydrophilic polymer. The peculiar aspect of this study is that the corrected isotherm indicates that the linear range of the isotherm is about $2x10^{-4}$ M Mg^{2+}, which is similar to that of the CPG-Phenanthroline system (Figure 3.5). However, the sorption at that point for CPG-Phenanthroline was about 0.4 μmol/g which corresponds to about 0.5% of the available capacity of the immobilized ligand. With all things being equal with the phenanthroline ligand, it could be expected that about

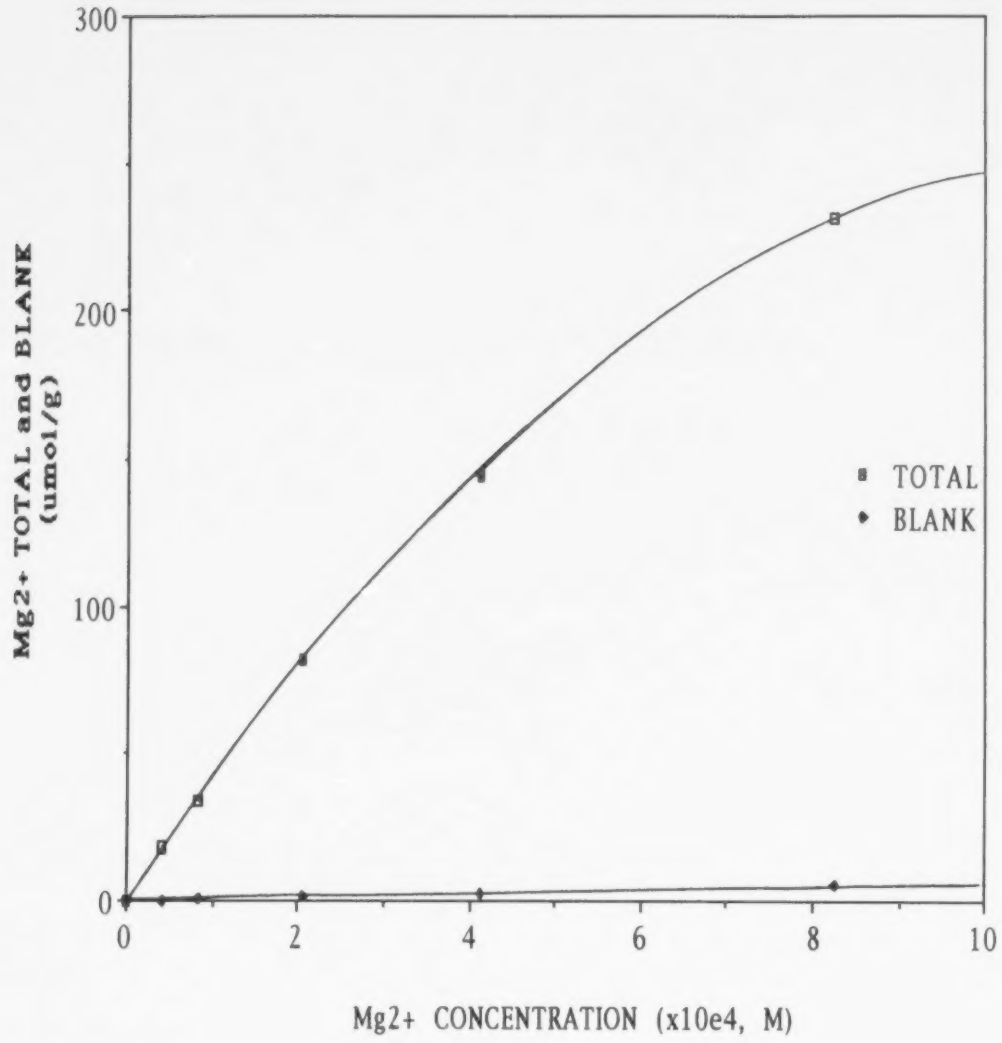


Figure 3.7. ISOTHERM / BLANK : P-PHENANTHROLINE

SAMPLES: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=7.00. BLANKS: 10% HNO_3. BOTH: X M Mg^{2+}; PROCEDURE: Water rinse, 2 min; Load solution, 15 min; Elute; Inject standards.

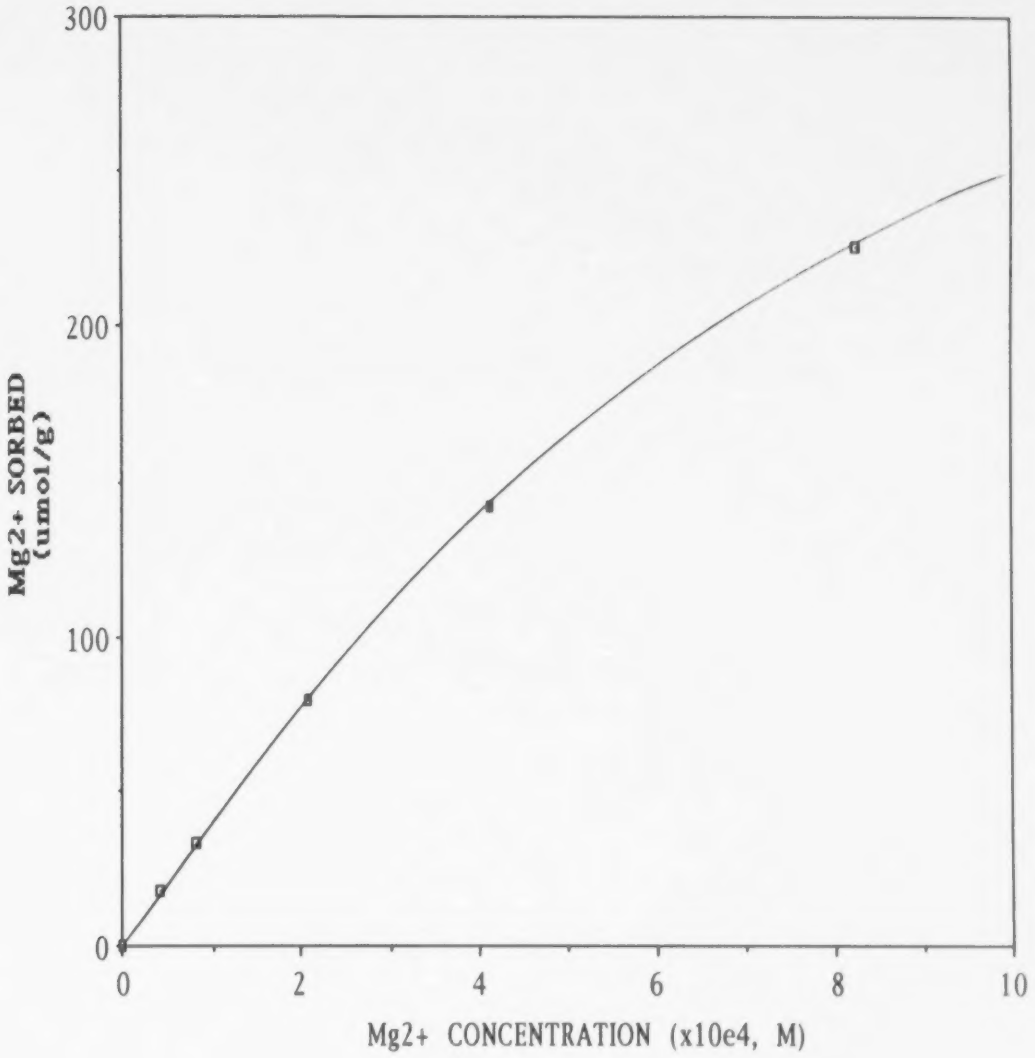


Figure 3.8. ISOTHERM : P-PHENANTHROLINE

This curve is obtained by subtracting the blank signal from the sample signal.

0.5% of the available capacity of the P-Phenanthroline would also be used for sorption under these conditions. Therefore, even though the sorption at this point (~ 80 μmol/g) for the P-Phenanthroline supposedly represents 100% of the available capacity, only a small percentage of the immobilized ligand capacity may actually be being used for sorption.

3.1.3.3 RECOMMENDATIONS

The results for this immobilized ligand suggests that further investigation should be done into the use of a hydrophilic polymer as the backbone for future immobilized ligands. In this case, it seems that the HEMA group itself is acting as an immobilized ligand and complexing Mg^{2+} ions based on its own distribution coefficient. Ideally, the backbone would be inert. Therefore, sorption studies on the HEMA backbone alone would be beneficial in the design of new backbone material. The identification of a hydrophilic backbone that was inert for these studies would be useful.

However, at this time, it was decided to switch to a hydrophobic backbone and attach a new ligand and carry out further studies. It seems necessary to study the hydrophobic case before performing an exhaustive study on hydrophilic supports. Also, it was decided to switch from the phenanthroline and investigate a new ligand since the values obtained for the linear range of the isotherms (2.5×10^{-4} M) were smaller than the desired millimolar range.

3.1.4 P-PHENYLGLYCINE

The polymer support used for this ligand is poly(STY/DVB). This polymer is hydrophobic in nature.

3.1.4.1 LOADING CURVE

An interesting development has occurred as evidenced by the appearance of Figure 3.9. As seen previously, a normal load study curve rises to a point and levels off at an equilibrium value. Not in this case. Up to 8 minutes, the curve looks normal and it even appears to level off. However, at longer load times, the curve continues to rise.

The continual rise at first suggests that the column has not come to equilibrium with the sample solution, even well after an hour. In an attempt to shorten the loading time, it was decided to try and precondition the column. This involves passing a solution containing the same ionic strength, buffer and pH through the column for a given amount of time, followed immediately by loading the Mg^{2+} containing solution. It was hoped that this preconditioning would bring the column to a state where the loading of the Mg^{2+} sample would be facilitated and therefore the loading time would decrease.

3.1.4.2 PRECONDITIONING STUDY

In this study, a sample solution was made to mimic the solution used in the loading curve study described in 3.1.4.1. However, the preconditioning

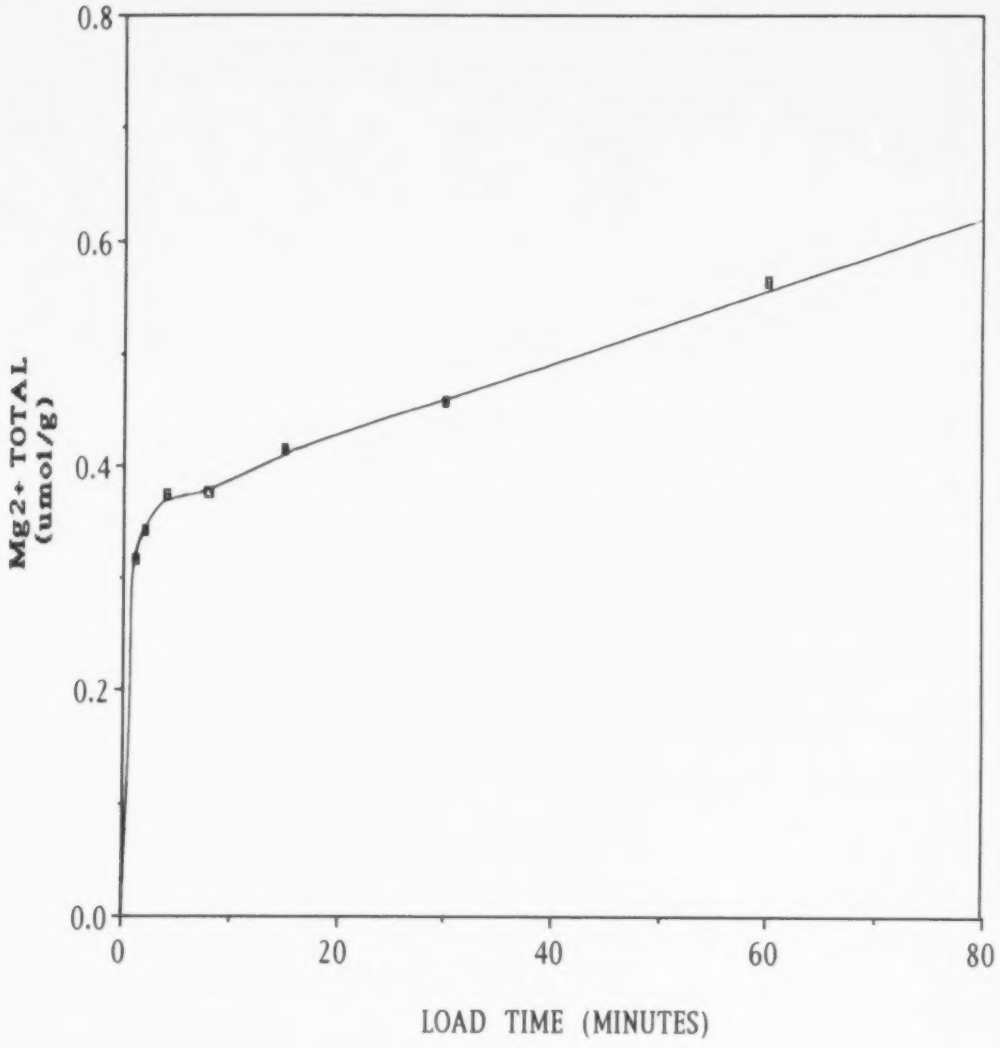


Figure 3.9. LOADING CURVE : P-PHENYLGLYCINE

SAMPLE: 4.1×10^{-5} M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Inject standards.

solution is made up with no Mg^{2+} added. Therefore, it would be expected that no Mg^{2+} signal would be observed. One of the first things to note about Figure 3.10 is that there is a definite Mg^{2+} signal. Therefore, there exists Mg^{2+} contribution from the reagents. This complication aside, the shape of this load curve resembles that of the sample load study in Figure 3.9.

Examination of these two curves reveals that they can almost be divided into two parts. The first part represents a normal load curve which rises and levels off. The second part is a straight line. It was noted that in previous work within the research group [11], it became necessary to pass the ionic strength adjusting component (NaCl) through a column of chelex to remove the Mg^{2+} impurities. In a separate study [9], Cu^{2+} impurities were removed by filtering the solutions through 0.45 μm pore filters. Presently, sample solutions are passed through a 40-60 μm filter. It was therefore decided to filter the solutions through a 0.45 μm filter and observe the results.

A similar sample solution containing no Mg^{2+} was prepared. However, this time prior to use in the column equilibration apparatus, the solution was passed through a 0.45 μm filter. The axes for Figure 3.11 are the same scale as used in Figure 3.10 for the unfiltered preconditioning load study. The effect of filtering is dramatic. There is no longer an appreciable Mg^{2+} signal and there is no continual rise in the data.

The observation of a continuous source of extra Mg^{2+} in the sample solution was actually unknowingly encountered in the load study of CPG-Phenanthroline (section 3.1.2.1). The reason for the curve not levelling off in that study can now be explained by the results from this section. This result was not obtained in the CPG-Oxine study (section 3.1.1.1) because the loading curve was not extended to long enough sample loading times. In the case of the P-Phenanthroline (section 3.1.3.1), the combination of the relatively large

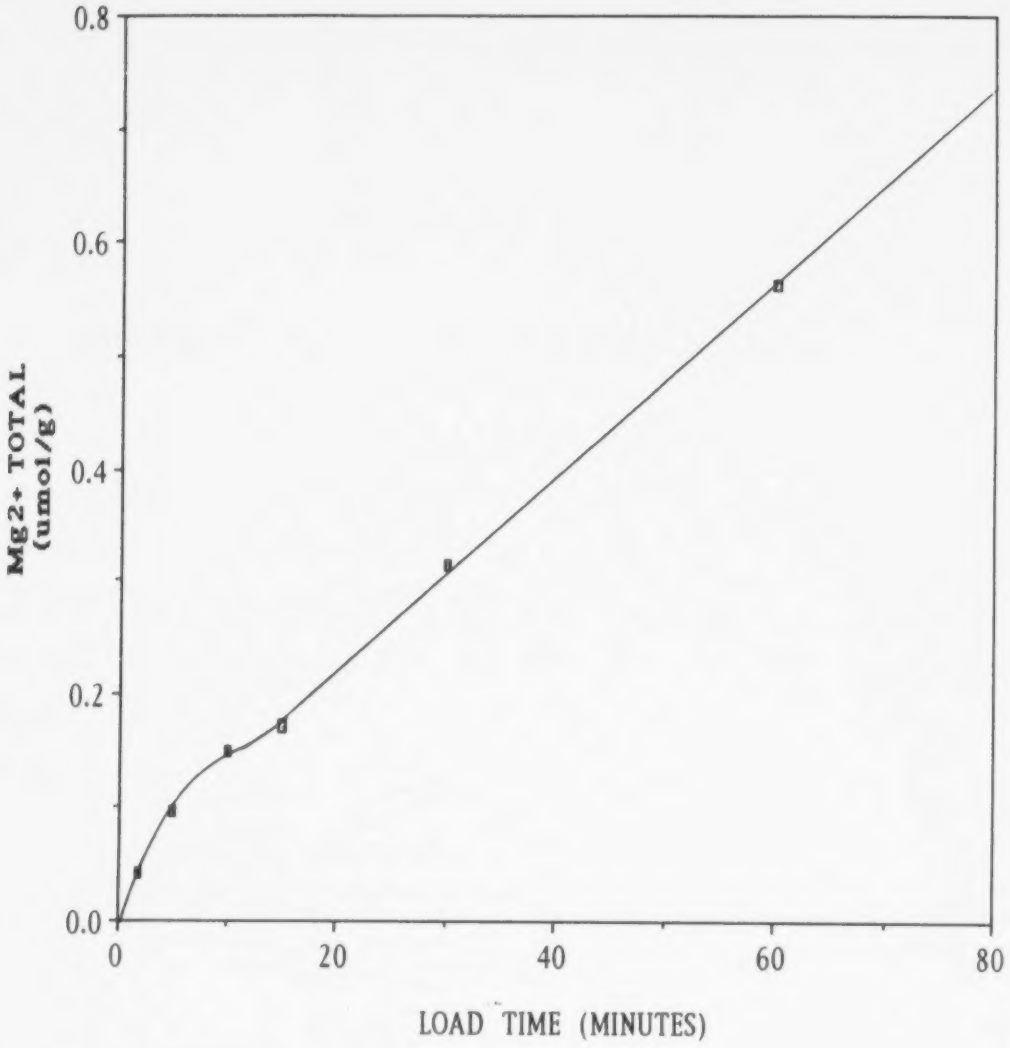


Figure 3.10. PRECONDITIONING LOADING CURVE : P-PHENYLGLYCINE

SAMPLE: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Inject standards.

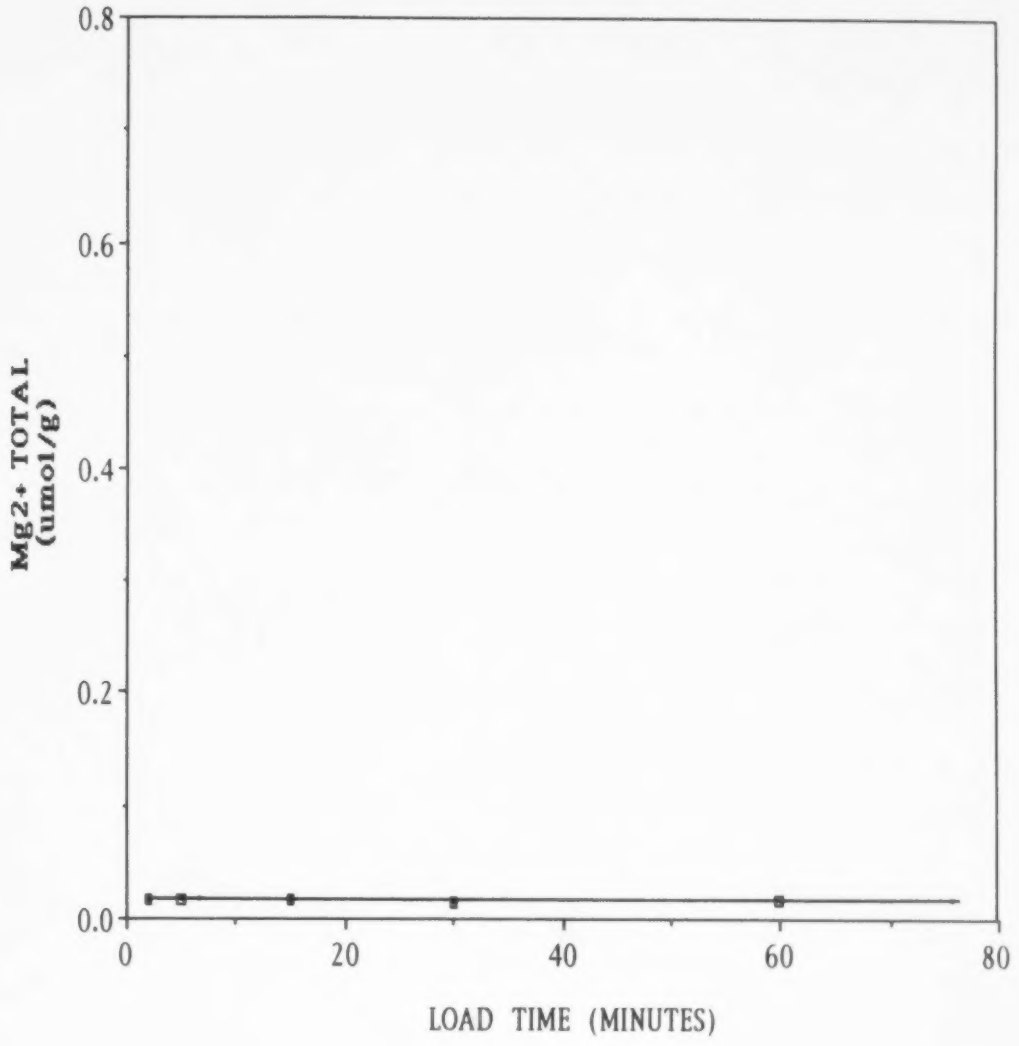


Figure 3.11. FILTERED PRECONDITIONING LOADING CURVE : P-PHENYLGLYCINE

SAMPLE: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Inject standards.

Mg^{2+} sorption signals obtained and again not extending the loading curve to long enough times, prevented the observation of the extra Mg^{2+}.

The filtering idea seems promising. However, whether or not a sample load curve would level off still remains to be seen. If the appearance of the leveling off portion in the initial sample load study was just an artifact, then filtering might not help the situation.

3.1.4.3 FILTERED LOAD STUDY

The Mg^{2+} loading curve was repeated using the conditions indicated in Figure 3.12. The results of the load study indicate that the time to reach equilibrium is about 10 minutes. After this time, the curve remains level. It should be pointed out that the vertical axis values in this filtered load study cannot be directly compared to the original unfiltered load study (3.1.4.1) since the Mg^{2+} concentrations are different.

Filtering the solutions through a 0.45 μm filter eliminated the Mg^{2+} contamination problem. If this is the actual case, then the impurity would have to be some insoluble magnesium compound. A thorough investigation into this source of magnesium was not undertaken. The source would have to be traced to the production and handling of the $NaClO_4$ prior to being shipped. For the purposes of future experiments, the identity of the contaminant was not a priority.

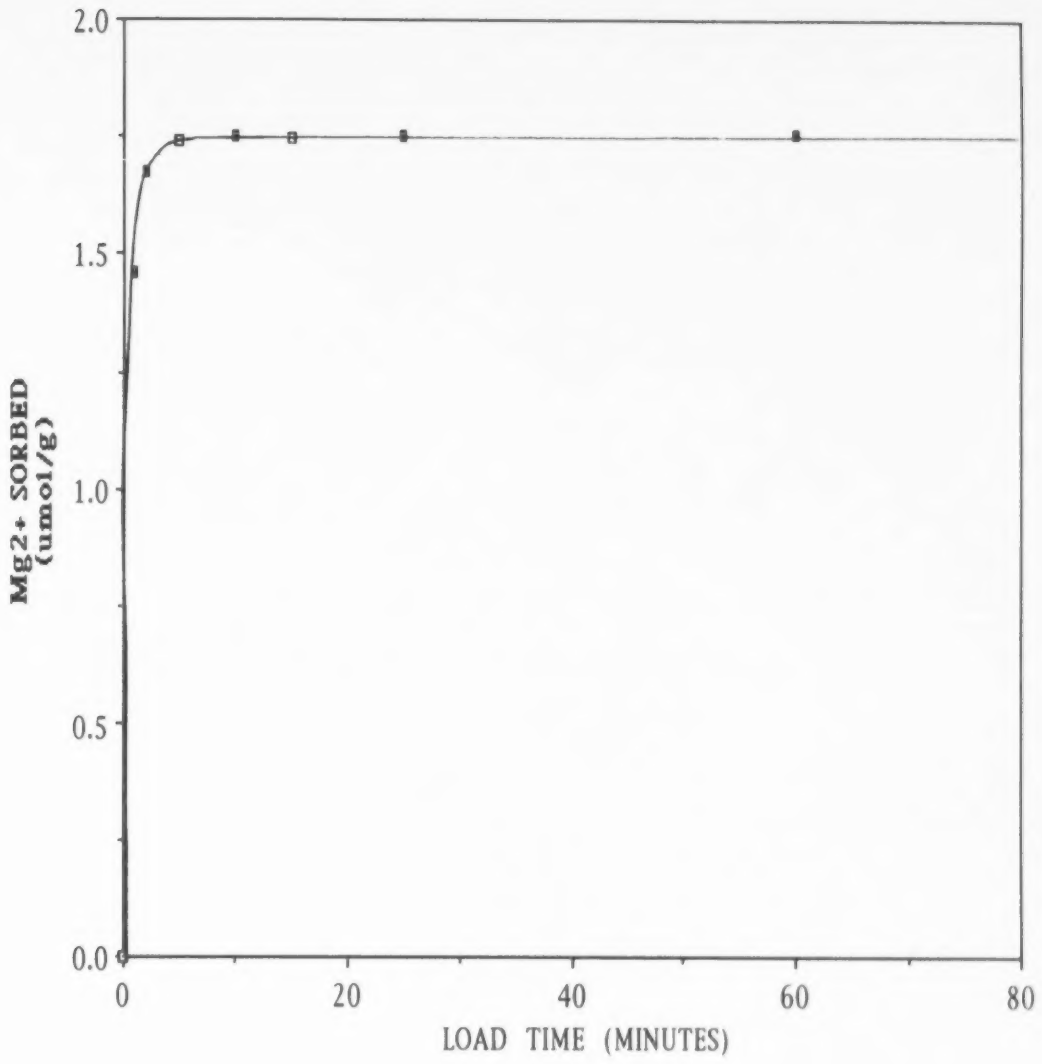


Figure 3.12. FILTERED LOADING CURVE : P-PHENYLGLYCINE

SAMPLE: $2x10^{-4}$ M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Inject standards.

3.1.4.4 ISOTHERM

The isotherm and blank study shown in Figure 3.13 indicates that the blank signal contributes about 30% to the total Mg^{2+} signal. This situation is not as severe as CPG-Phenanthroline case and it is acceptable to subtract the data and obtain a reliable value. The corrected isotherm is shown in Figure 3.14and it gives linearity up to about $5x10^{-4}$ M Mg^{2+}.

3.1.4.5 RECOMMENDATIONS

The P-Phenylglycine system performs in a well-behaved manner. The load time is reasonable so that large quantities of solutions are not required and the isotherm shows linearity into the range of Mg^{2+} ions found in biological fluids. However, since there is a noticeable blank signal, it was decided to try a different immobilized ligand.

The new ligand chosen has a slightly higher formation constant with Mg^{2+} than glycine and also a higher capacity. It is hoped that this adjustment will result in more Mg^{2+} ions being sorbed at equilibrium which will increase the difference between the sorbed and blank Mg^{2+} signals. It should be noted that P-Phenylglycine is not being abandoned. It produced acceptable results and will be examined further for its specificity properties in the upcoming sections.

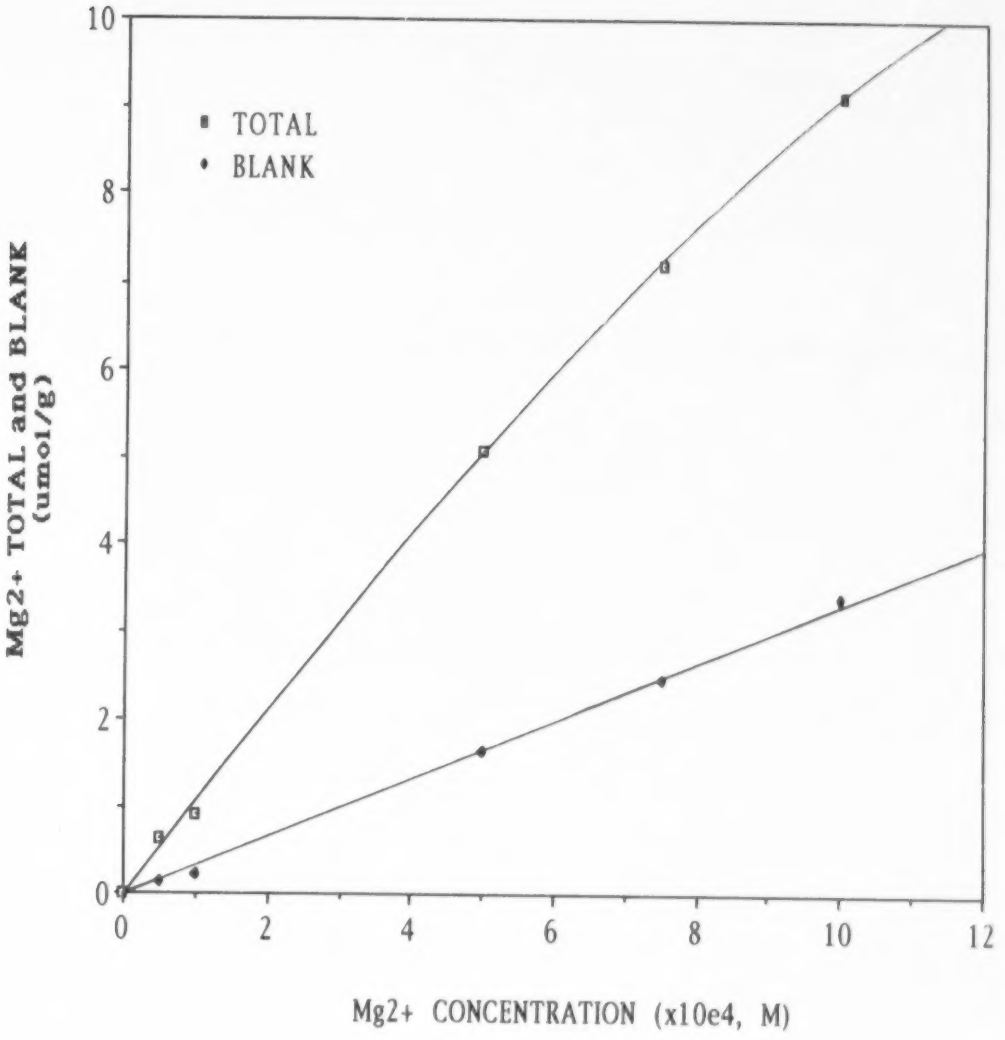


Figure 3.13. ISOTHERM / BLANK : P-PHENYLGLYCINE

SAMPLES: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. BLANKS: 10% HNO_3. BOTH: X M Mg^{2+}. PROCEDURE: Water rinse, 2 min; Load solution, 10 min; Elute; Inject standards.

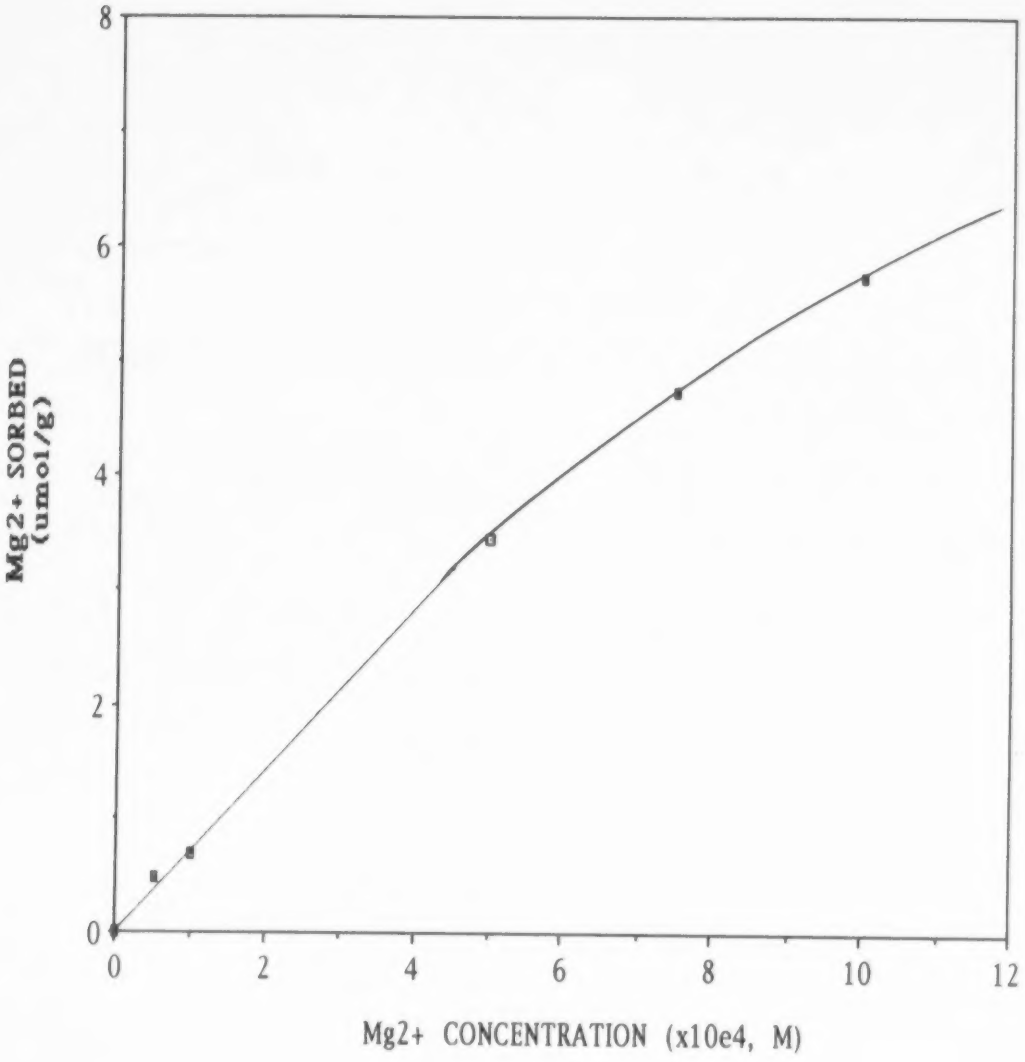


Figure 3.14. ISOTHERM : P-PHENYLGLYCINE

This curve is obtained by subtracting the blank signal from the sample signal.

3.1.5 P-PICOLINIC ACID

3.1.5.1 LOADING CURVE

The load time for this system (Figure 3.15) is similar to that found for the P-Phenylglycine system. As seen, the curve levels off after about 10 minutes. It should also be noted that the Y-axis values are larger in this case than for P-Phenylglycine. This can be attributed to the higher capacity and larger formation constant.

3.1.5.2 TYPICAL CALIBRATION CURVE

As a result of the higher signals obtained with this system, the eluted sample must be collected in a vial and diluted prior to being aspirated into the AAS. The signals obtained by eluting directly into the AAS are beyond the linearity of the AAS and are therefore not usable. In all of the previous immobilized ligand studies, the observed Mg^{2+} signals were within the linearity of the AAS and direct elution into the AAS (Figure 2.6) was used. Figure 3.16 shows a typical Mg^{2+} calibration curve used to quantitate the Mg^{2+} levels collected in the vials. Since an extra step is involved in this type of system, it is critical that the collection be well controlled. The vials must be thoroughly cleaned and the collected sample weights must be determined accurately. The calculations assume a solution density of 1 g/mL.

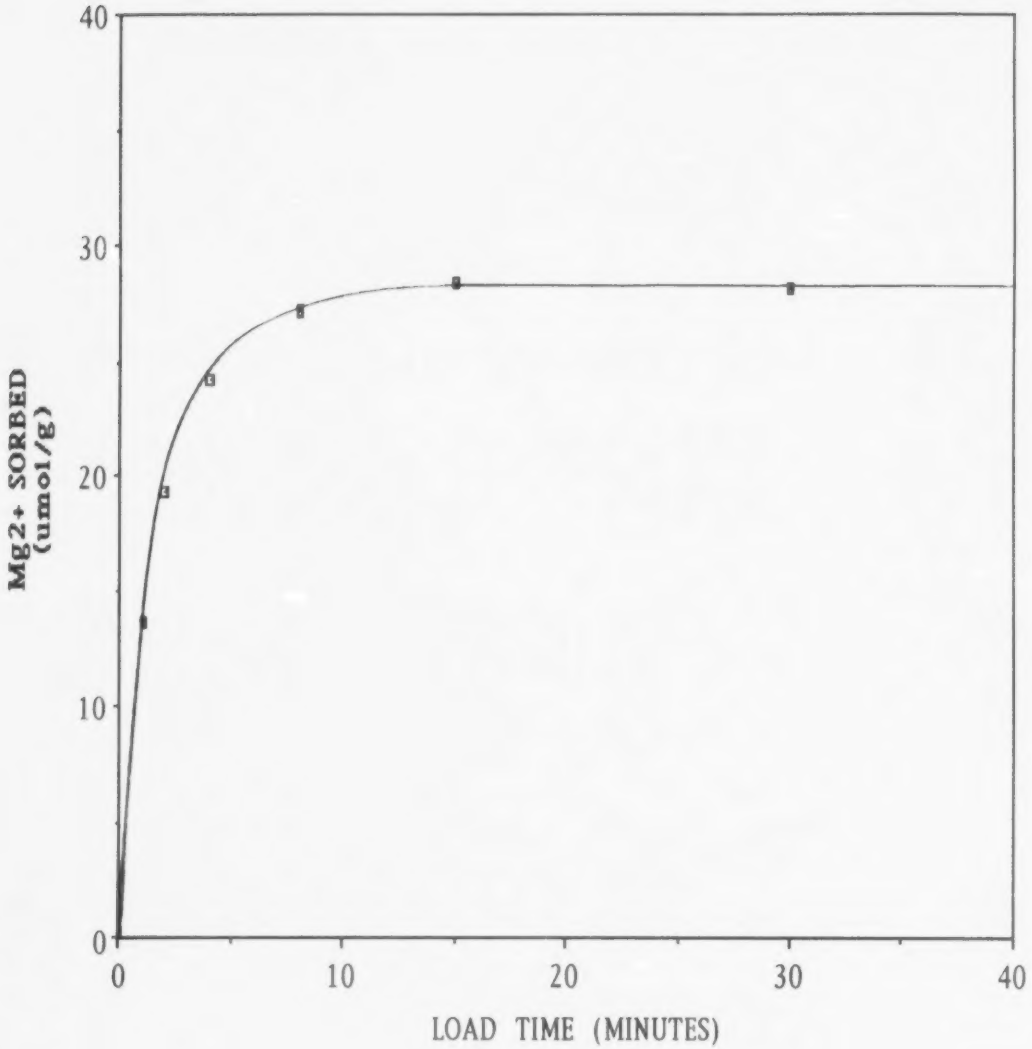


Figure 3.15. LOADING CURVE : P-PICOLINIC ACID

SAMPLE: 1.0×10^{-4} M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load sample, X min; Elute; Collect in a vial; Analyze.

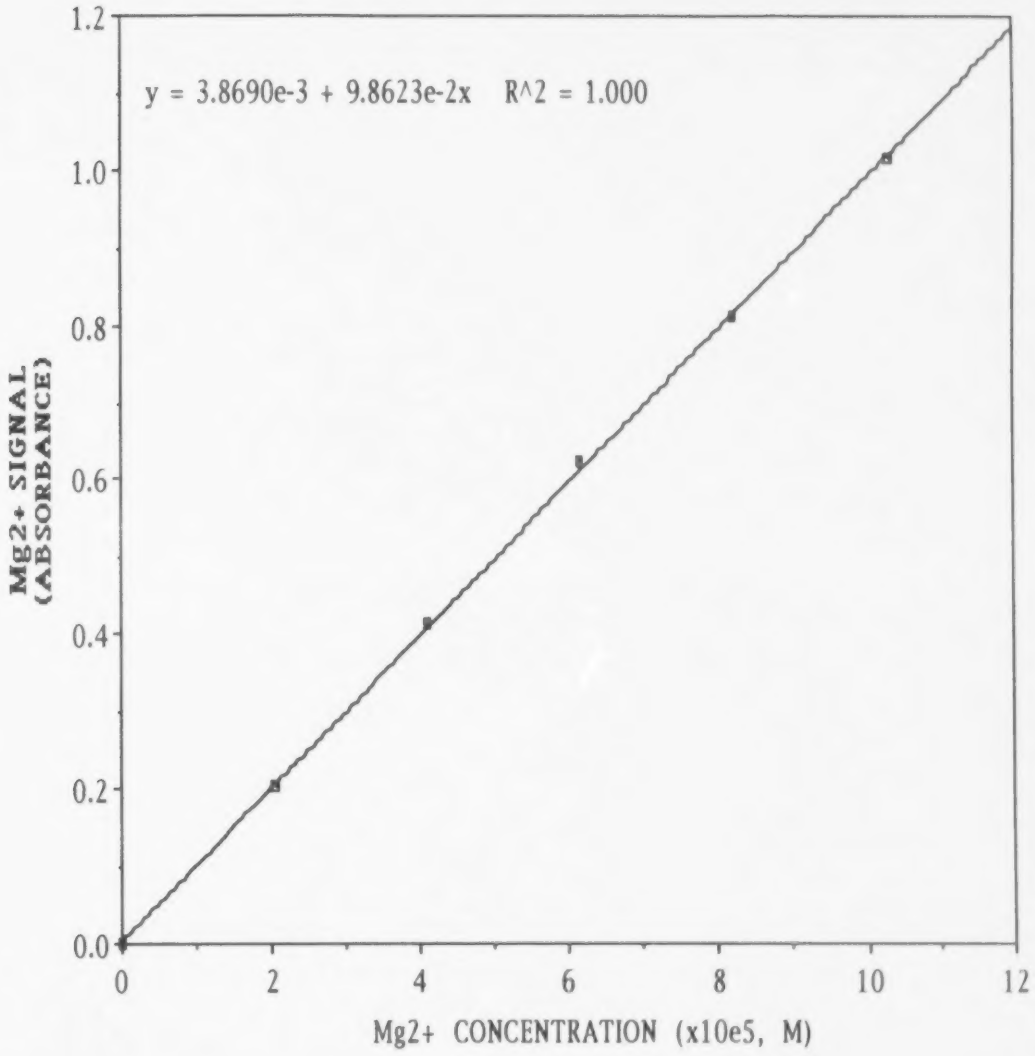


Figure 3.16. TYPICAL CALIBRATION CURVE

SAMPLES: X M Mg^{2+}; 1% HNO_3; PROCEDURE: Direct aspiration of 1% HNO_3 to establish baseline; Aspirate sample; Record absorbance value.

3.1.5.3 ISOTHERM

The isotherm for this system is shown in Figure 3.17. The distribution coefficient is constant up to about $2x10^{-4}$ M Mg^{2+}. A hold-up volume study revealed that the blank signals were very small (~3%) compared to the total signal of the sorbed Mg^{2+} from the sample.

3.1.5.4 RECOMMENDATIONS

The linear sample capacity is not as high in this system as it is for the P-Phenylglycine system. However, the large sample signals and the low blank signals are an enticing combination for further studies. Recall that P-Phenanthroline had this combination but the system was not investigated further since it seemed that extra sorption was occurring. In the P-Picolinic acid case, the signal for $2x10^{-4}$ M Mg^{2+} represents only about 6% of the total capacity of the immobilized ligand.

3.1.6 SUMMARY

In this section, the loading curves and isotherms of 5 separate immobilized ligands have been investigated. It has been shown that these immobilized ligands do not behave the same way with respect to the sorption of Mg^{2+} ions from solution. It is these differences, and more specifically the factors which cause these differences, that have been examined here. The nature of the solid support, the formation constant between the ligand and Mg^{2+} ion and the capacity of the immobilized ligand for Mg^{2+} sorption have

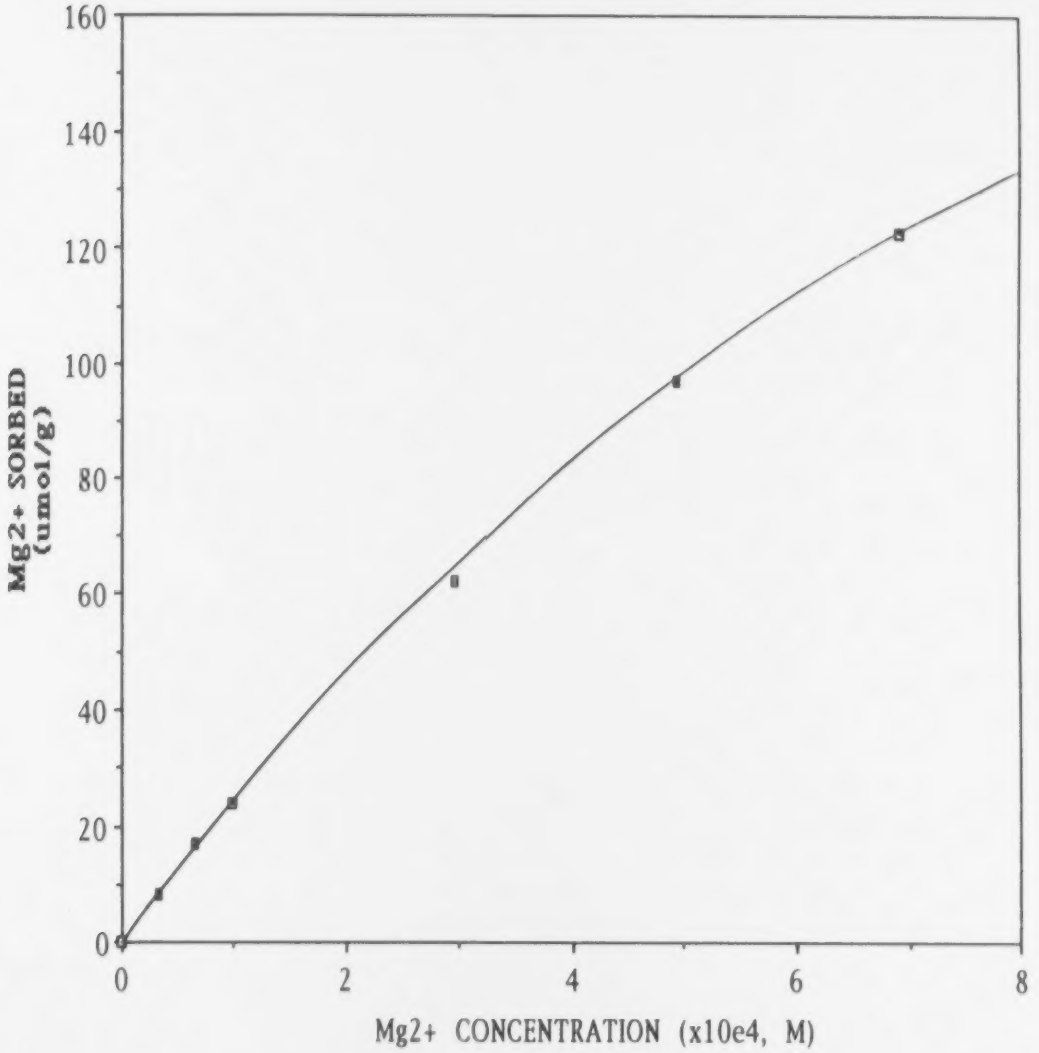


Figure 3.17. ISOTHERM : P-PICOLINIC ACID

SAMPLES: X M Mg^{2+}; 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load sample, 8 min; Elute; Collect in a vial; Analyze.

been shown to affect both the relative magnitude of the hold-up volume or blank signals obtained compared to the sorbed signals and also the linear sample capacity.

The commercial CPG-Oxine had too high a formation constant and as a result, the linear range extended only to about 2.5×10^{-5} M Mg^{2+} since the column quickly became overloaded.

The phenanthroline ligand was then attached to CPG and investigated since it had a smaller formation constant. However, the combination of a small formation constant and a low capacity resulted in extremely low sorption so that the contribution from the hold-up volume was relatively very high. It was concluded that a higher capacity solid support was needed.

The phenanthroline ligand was then attached to a hydrophilic polymer support. The capacity was increased by 80 fold. The sorbed signals did increase dramatically and the blank signal was no longer a problem. However, the sorption was too high. Calculations showed that the capacity of the immobilized ligand was exceeded and this could presumably be attributed to sorption of Mg^{2+} ions by the polymer itself. This assumption remains to be tested experimentally.

A hydrophobic polymer support was chosen for attachment of the glycine ligand. This system had a small formation constant. As a result, even with the high capacity, the relative contribution from the hold-up volume was measurable. However, the relative blank signal was not too large and the isotherm was linear up to about 5×10^{-4} M Mg^{2+} . This system was considered to hold potential for use in the measurement of free magnesium ion concentration and was therefore chosen for further investigation (section 3.2).

Picolinic acid was then tried on a similar hydrophobic polymer with a similar capacity since its formation constant is slightly larger than the glycine constant. This did greatly reduce the blank contribution and afforded high signals. The linear range was up to about $2x10^{-4}$ M Mg^{2+} and this system was also chosen for further studies.

As can be seen from these investigations, the factors that contribute to a useful immobilized ligand can be controlled and manipulated. A progression of ideas has led to improvements from the basic commercial CPG-Oxine to the P-Picolinic acid produced in-house. Before further improvements can be made, it is necessary to study the more promising systems in more detail.

3.2 SPECIFICITY OF IMMOBILIZED LIGANDS FOR Mg^{2+}

The underlying idea for these studies is to determine the free metal ion concentration in the presence of metal-ligand complexes. Therefore, the complexing ligands EDTA, picolinic acid and oxalic acid were chosen since it is desirable to do this investigation in the presence of negative, positive and neutral metal-ligand species. By adding different limiting (substoichiometric) amounts of a complexing ligand to a fixed total metal concentration, sample solutions having varying ratios of complexed to uncomplexed metal ions can be obtained. These experiments are designed in such a way that it is known what concentration of metal ion is uncomplexed. Therefore, appropriate standard solutions with matching metal ion concentrations can be made. If the immobilized ligand is specific for the metal ion then the signals for the sample and standard will be identical. The standard solutions are made up of known amounts of Mg^{2+}, ionic strength adjuster ($NaClO_4$) and HEPES buffer.

The sample solutions contain the same components plus the addition of the complexing ligand. The column equilibration apparatus (Figures 2.6 and 2.7) are used for these studies.

3.2.1 EDTA AS SOLUBLE LIGAND

In this section, the following notations are used :

a) IONS IN SOLUTION : Mg^{2+}, H^{+}, Na^{+}, Y^{4-}, HY^{3-}

b) COMPLEXES IN SOLUTION : MgY^{2-}, $MgHY^{-}$, HY^{3-}, NaY^{3-}

c) FORMATION CONSTANTS : K_1, K_2, K_X, K_N, K_H

d) FRACTION OF THE GIVEN SPECIES IN SOLUTION : α_{Mg2+}, α_{Y4-}, α_{HY3-}

e) CONDITIONAL FORMATION CONSTANTS : K_1', K_X', K_N'

f) TOTAL CONCENTRATIONS ADDED TO SOLUTION : C_{Mg}, C_Y

g) TOTAL CONCENTRATION OF UNCOMPLEXED EDTA : $[Y']$

EDTA forms two complexes with Mg^{2+} : MgY^{2-} and $MgHY^{-}$. The formation expressions for these two complexes as given in Smith and Martell [51] are as follows :

$$Mg^{2+} + Y^{4-} \Leftrightarrow MgY^{2-} \qquad K_1 = \frac{[MgY^{2-}]}{[Mg^{2+}][Y^{4-}]} \tag{3.3}$$

$$MgY^{2-} + H^{+} \Leftrightarrow MgHY^{-} \qquad K_2 = \frac{[MgHY^{-}]}{[MgY^{2-}][H^{+}]} \tag{3.4}$$

For the purposes of the following specificity studies, it is necessary that both expressions appear as complexation reactions with Mg^{2+}. The first expression

satisfies this requirement. However, the second expression needs to be changed so that it has the form of METAL + LIGAND ⇔ COMPLEX. To do this derivation, another expression is needed :

$$H^+ + Y^{4-} \Leftrightarrow HY^{3-} \qquad K_H = \frac{[HY^{3-}]}{[H^+][Y^{4-}]} \tag{3.5}$$

From K_2

$$MgY^{2-} = \frac{[MgHY^-]}{K_2[H^+]} \tag{3.6}$$

From K_H

$$[Y^{4-}] = \frac{[HY^{3-}]}{K_H[H^+]} \tag{3.7}$$

Therefore, equation 3.3 can be rewritten as

$$K_1 = \frac{[MgHY^-]}{K_2[H^+]} \times \frac{1}{[Mg^{2+}]} \times \frac{K_H[H^+]}{[HY^{3-}]} \tag{3.8}$$

which simplifies to

$$\frac{K_1 \cdot K_2}{K_H} = \frac{[MgHY^-]}{[Mg^{2+}] \cdot [HY^{3-}]} = K_X \tag{3.9}$$

In order to do the specificity studies, it is necessary to be able to calculate the species distribution of magnesium in a solution containing EDTA. Therefore, the following is a derivation of an expression which enables this calculation to be made. From it, the concentration of EDTA that must be added to a solution containing a given concentration of magnesium to result in a specified amount of uncomplexed and complexed Mg^{2+} ions can be calculated. Because of the presence of sodium in these studies, it is necessary to include the effect of its complexation by EDTA.

$$Mg^{2+} + Y^{4-} \Leftrightarrow MgY^{2-} \tag{3.10}$$

$$K_1 = \frac{[MgY^{2-}]}{[Mg^{2+}] \cdot [Y^{4-}]} \tag{3.11}$$

$$K_1' = K_1 \cdot \alpha_{Y^{4-}} = \frac{[MgY^{2-}]}{[Mg^{2+}] \cdot [Y']} \tag{3.12}$$

$$Mg^{2+} + HY^{3-} \Leftrightarrow MgHY^{-} \tag{3.13}$$

$$K_X = \frac{[MgHY^{-}]}{[Mg^{2+}] \cdot [HY^{3-}]} \tag{3.14}$$

$$K_X' = K_X \cdot \alpha_{HY^{3-}} = \frac{[MgHY^{-}]}{[Mg^{2+}] \cdot [Y']} \tag{3.15}$$

$$Na^{+} + Y^{4-} \Leftrightarrow NaY^{3-} \tag{3.16}$$

$$K_N = \frac{[NaY^{3-}]}{[Na^{+}] \cdot [Y^{4-}]} \tag{3.17}$$

$$K_N' = K_N \cdot \alpha_{Y^{4-}} = \frac{[NaY^{3-}]}{[Na^{+}] \cdot [Y']} \tag{3.18}$$

These expressions can then be used to relate the uncomplexed concentration of Mg^{2+} in solution to the total concentration of magnesium added to the solution.

$$\alpha_{Mg^{2+}} = \frac{[Mg^{2+}]}{C_{Mg}} \tag{3.19}$$

$$\alpha_{Mg^{2+}} = \frac{[Mg^{2+}]}{[Mg^{2+}] + [MgY^{2-}] + [MgHY^{-}]} \tag{3.20}$$

$$\alpha_{Mg^{2+}} = \frac{[Mg^{2+}]}{[Mg^{2+}] + (K_1' \cdot [Mg^{2+}] \cdot [Y']) + (K_X' \cdot [Mg^{2+}] \cdot [Y'])} \quad (3.21)$$

$$\alpha_{Mg^{2+}} = \frac{1}{1 + (K_1' \cdot [Y']) + (K_X' \cdot [Y'])} \quad (3.22)$$

$$\alpha_{Mg^{2+}} = \frac{1}{1 + (K_1' + K_X') \cdot [Y']} \quad (3.23)$$

Since

$$[Y'] = C_Y - [MgY^{2-}] - [MgHY^-] - [NaY^{3-}] \quad (3.24)$$

and

$$[MgHY^-] = C_{Mg} - [Mg^{2+}] - [MgY^{2-}] \quad (3.25)$$

therefore

$$[Y'] = C_Y - [MgY^{2-}] - C_{Mg} + [Mg^{2+}] + [MgY^{2-}] - [NaY^{3-}] \quad (3.26)$$

After substituting for $[NaY^{3-}]$ from eq. 3.18, this can be rearranged to give :

$$[Y'] + (K_N' \cdot [Na^+] \cdot [Y']) = C_Y - C_{Mg} + [Mg^{2+}] \quad (3.27)$$

which leads to:

$$[Y'] = \frac{C_Y - C_{Mg} + [Mg^{2+}]}{(1 + K_N' \cdot [Na^+])} \quad (3.28)$$

This expression can be substituted into equation (3.23) to obtain :

$$\alpha_{Mg^{2+}} = \frac{1}{1 + (K_1' + K_X') \cdot \left(\frac{C_Y - C_{Mg} + [Mg^{2+}]}{(1 + K_N' \cdot [Na^+])} \right)} \quad (3.29)$$

and this expression can be rearranged into the following :

$$C_Y = \frac{\left(\frac{1}{\alpha_{Mg^{2+}}} - 1\right)}{\left(K_1' + K_X'\right)} \cdot \left(1 + K_N' \cdot [Na^+]\right) + C_{Mg} - [Mg^{2+}] \qquad (3.30)$$

This equation might seem a little complicated but it is not. C_{Mg} can be selected and α_{Mg2+} can be specified. This then fixes $[Mg^{2+}]$. The $[Na^+]$ is actually calculated after all of the reagents have been added to the solution.

The only part of the equation that involves extensive work is the calculation of the conditional formation constants. In order to do this, all of the relevant literature K values, which are quoted at specific ionic strengths, must be corrected to the ionic strength (I) to be used in these studies (Table 3.2a). This involves calculating the activity coefficients (γ) of all of the necessary ions. This is accomplished using the Davies' equation [53]:

$$-\log(\gamma_\pm) = \left(0.5 \cdot z^2\right)\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3 \cdot I\right) \qquad (3.31)$$

where z is the charge on the ion. For the general equation A + B → C, the K values can then be adjusted with the following equation :

$$K_{NEW} = K_{LIT} \cdot \frac{\gamma_{C,LIT}}{\gamma_{A,LIT} \cdot \gamma_{B,LIT}} \cdot \frac{\gamma_{A,NEW} \cdot \gamma_{B,NEW}}{\gamma_{C,NEW}} \qquad (3.32)$$

In order to obtain the conditional formation constant values, it is necessary to calculate the species distribution of EDTA at the given ionic strength and pH. The total concentration of uncomplexed EDTA in solution can be represented by the following expression :

$$[Y'] = [Y^{4-}] + [HY^{3-}] + [H_2Y^{2-}] + [H_3Y^-] + [H_4Y] + [H_5Y^+] + [H_6Y^{2+}] \qquad (3.33)$$

REACTION	log K_{LIT} (25°C,0.1 M)	log K_{NEW} (25°C,0.2 M)	FRACTION OF SPECIES (pH=6) (25°C,0.2 M)
$H^+ + Y^{4-} \Leftrightarrow HY^{3-}$	10.24	10.10	$\alpha_{Y4-} = 3.65 \times 10^{-5}$
$H^+ + HY^{3-} \Leftrightarrow H_2Y^{2-}$	6.16	6.06	$\alpha_{HY3-} = 0.46$
$H^+ + H_2Y^{2-} \Leftrightarrow H_3Y^-$	2.66	2.60	$\alpha_{H2Y2-} = 0.54$
$H^+ + H_3Y^- \Leftrightarrow H_4Y$	2.00	1.98	$\alpha_{H3Y-} = 2.14 \times 10^{-4}$
$H^+ + H_4Y \Leftrightarrow H_5Y^+$	1.50	1.52	$\alpha_{H4Y} = 2.05 \times 10^{-8}$
			$\alpha_{H5Y+} = 6.78 \times 10^{-13}$

REACTION	log K_{LIT} (25°C,0.1 M)	log K_{NEW} (25°C,0.2 M)	log K' (pH=6) (25°C,0.2 M)
$Mg^{2+} + Y^{4-} \Leftrightarrow MgY^{2-}$	8.79	8.48	4.04
$MgY^{2-} + H^+ \Leftrightarrow MgHY^-$	3.85	-	-
$Mg^{2+} + HY^{3-} \Leftrightarrow MgHY^-$	-	2.17	1.83
$Na^+ + Y^{4-} \Leftrightarrow NaY^{3-}$	1.66	1.50	-2.93

Table 3.2. (a) Top - Protonation values for EDTA
(b) Bottom - Complexation values for Mg-EDTA

Each of the terms in equation 3.33 can be represented by expressions for their equilibrium formation constants, K_{HaYb} (where a and b are small whole numbers). Therefore, equation 3.33 can be rewritten in a different manner :

$$[Y'] = 1 + [H^+]\cdot K_{HY^{3-}} + [H^+]^2\cdot K_{H_2Y^{2-}}\cdot K_{HY^{3-}} + [H^+]^3\cdot K_{H_3Y^-}\cdot K_{H_2Y^{2-}}\cdot K_{HY^{3-}}$$
$$+ [H^+]^4\cdot K_{H_4Y}\cdot K_{H_3Y^-}\cdot K_{H_2Y^{2-}}\cdot K_{HY^{3-}} + [H^+]^5\cdot K_{H_5Y^+}\cdot K_{H_4Y}\cdot K_{H_3Y^-}\cdot K_{H_2Y^{2-}}\cdot K_{HY^{3-}}$$
$$+ [H^+]^6\cdot K_{H_6Y^{2+}}\cdot K_{H_5Y^+}\cdot K_{H_4Y}\cdot K_{H_3Y^-}\cdot K_{H_2Y^{2-}}\cdot K_{HY^{3-}} \qquad (3.34)$$

Therefore, the fraction of EDTA in the form Y^{4-} can be represented by the expression $1/[Y']$ (the first term in equation 3.34 divided by $[Y']$). The fraction of EDTA in the form HY^{3-} can be represented by the expression $([H^+]\ K_{HY3-})/[Y']$ (the second term in equation 3.34 divided by $[Y']$). After calculating the fractions of the different EDTA species present under the given pH conditions, the appropriate conditional constants, K', values can then be obtained (Table 3.2b). For example, $K'_{MgY^{2-}} = K_{MgY^{2-}} * \alpha_{Y^{4-}}$.

There are numerous tedious calculations in this process. In the best interests of time and accuracy, a computer program has been written which performs all these calculations. The program appears in appendix B1. The program uses the literature K values and calculates the following :

a) the K values at a new specified I
b) the species distribution of EDTA at the specified pH
c) the K' values for the metal-EDTA complexes
d) the fractions of Mg^{2+}, MgY^{2-} and $MgHY^-$ in a solution of a given C_{Mg}, C_Y and $[Na^+]$.

The program is a fast and effective way of determining a desired set of experimental conditions.

The specificity experiments for Mg^{2+} in the presence of EDTA will be examined using the P-Picolinic acid and P-Phenylglycine immobilized ligand systems. These studies will determine if the immobilized ligands are specific for Mg^{2+} in the presence of its negative metal-ligand complexes (MgY^{2-} and $MgHY^{-}$).

3.2.1.1 P-PICOLINIC ACID IMMOBILIZED LIGAND

As seen in Figure 3.18, there is good agreement between the standard and sample data for the determination of Mg^{2+} ions in the presence of EDTA for the P-Picolinic acid system. This agreement is consistent over the entire range of solutions tested. At high concentrations of EDTA added (meaning a lower concentration of uncomplexed Mg^{2+} ions in solution), the agreement is as good as at the other end where the concentration of Mg^{2+} ions in solution is higher (meaning a lower concentration of EDTA added) (Table 3.3a). This excellent result leads to the conclusion that this system is specific for Mg^{2+} ions in the presence of its negative ligand complexes.

3.2.1.2 P-PHENYLGLYCINE AS IMMOBILIZED LIGAND

The results for the P-Phenylglycine system (Figure 3.19) are as successful as in the P-Picolinic acid case. As seen, the agreement between the standard and sample data is excellent over the entire range of solutions tested

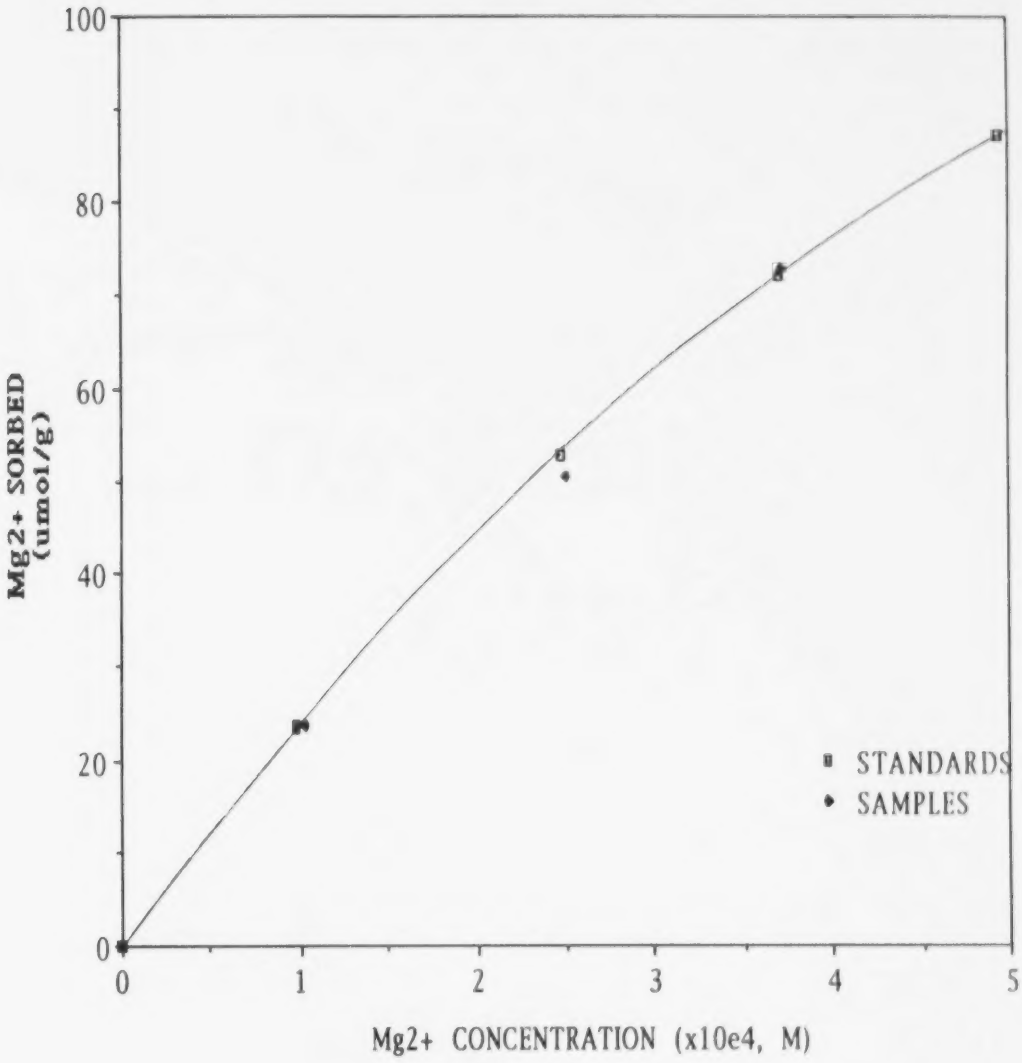


Figure 3.18. EDTA SPECIFICITY : P-PICOLINIC ACID

SAMPLES: 4.9×10^{-4} M Mg^{2+}; X M EDTA. STANDARDS: Y M Mg^{2+}. BOTH: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load solution, 15 min; Elute; Collect in a vial; Analyze.

[Mg] TOTAL ADDED ($x10^{-4}$ M)	[EDTA] TOTAL ADDED ($x10^{-4}$ M)	pH	α Mg^{2+}	α MgY^{2-}	α $MgHY^{-}$	pMg CALC.	pMg EXPT.
4.94	7.33	6.00	0.209	0.786	0.005	3.99	4.00
4.94	3.31	6.00	0.507	0.490	0.003	3.60	3.63
4.94	1.52	6.00	0.753	0.246	0.002	3.43	3.43

[Mg] TOTAL ADDED ($x10^{-4}$ M)	[EDTA] TOTAL ADDED ($x10^{-4}$ M)	pH	α Mg^{2+}	α MgY^{2-}	α $MgHY^{-}$	pMg CALC.	pMg EXPT.
5.00	6.40	6.00	0.253	0.742	0.005	3.90	3.87
5.00	3.20	6.00	0.524	0.473	0.003	3.58	3.59
5.00	1.60	6.00	0.743	0.256	0.002	3.43	3.43

Table 3.3. (a) Top - Mg^{2+} specificity in the presence of Mg-EDTA complexes using the P-Picolinic acid system
(b) Bottom - Mg^{2+} specificity in the presence of Mg-EDTA complexes using the P-Phenylglycine system

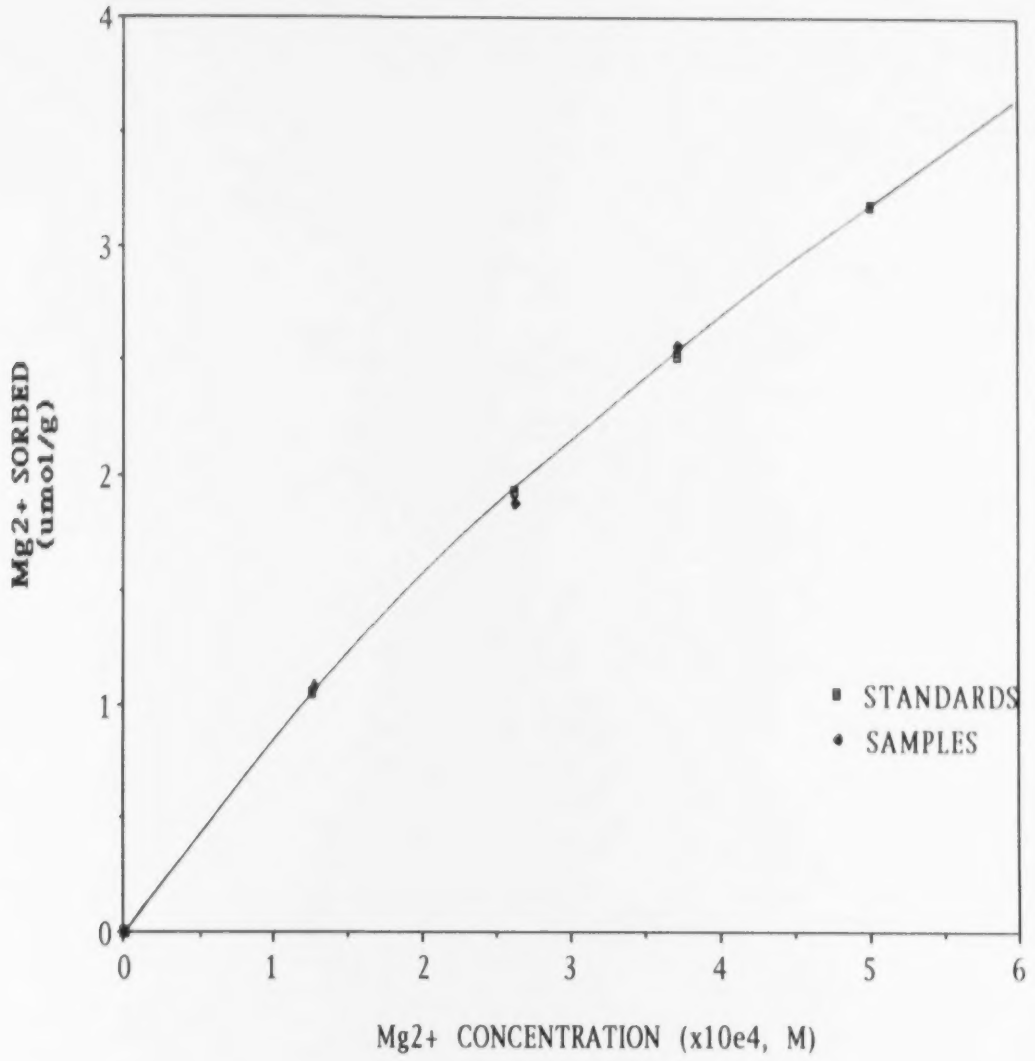


Figure 3.19. EDTA SPECIFICITY : P-PHENYLGLYCINE

SAMPLES: $5x10^{-4}$ M Mg^{2+}; X M EDTA. STANDARDS: Y M Mg^{2+}. BOTH: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load solution, 10 min; Elute; Inject standards.

(Table 3.3b). Since no extra sorption is occurring, neither the immobilized ligand nor the polymer backbone is sorbing any complexes.

3.2.2 PICOLINIC ACID AS SOLUBLE LIGAND

In this section, the following notations are used :

a) IONS IN SOLUTION : Mg^{2+}, P^-

b) COMPLEXES IN SOLUTION : MgP^+, MgP_2

c) FORMATION CONSTANTS : β_1, β_2

d) FRACTION OF SPECIES IN THE GIVEN FORM : α_{Mg2+}, α_{P-}

e) CONDITIONAL FORMATION CONSTANTS : β_1', β_2'

f) TOTAL CONCENTRATIONS ADDED TO SOLUTION : C_{Mg}, C_P

g) TOTAL CONCENTRATION OF UNCOMPLEXED PICOLINIC ACID : $[P']$

Picolinic acid (P) forms two complexes with Mg^{2+} : MgP^+ and MgP_2. The formation expressions for these two complexes as given in Smith and Martell [51] are as follows :

$$Mg^{2+} + P^- \Leftrightarrow MgP^+ \qquad \beta_1 = \frac{[MgP^+]}{[Mg^{2+}][P^-]} \tag{3.35}$$

$$Mg^{2+} + 2P^- \Leftrightarrow MgP_2 \qquad \beta_2 = \frac{[MgP_2]}{[Mg^{2+}][P^-]^2} \tag{3.36}$$

In order to do the specificity studies, it is necessary to be able to calculate the species distribution of magnesium in a solution containing picolinic acid. As in the EDTA case, conditional formation constants must be

calculated. Again, this involves converting the literature values to the appropriate constants at the required ionic strengths using equations 3.31 and 3.32 (Table 3.4a). These "new" formation constants can then be converted to conditional values by determining the fraction of the appropriate picolinic acid species which is present in the system at the given pH.

To begin with, the total concentration of uncomplexed picolinic acid in solution can be represented by the following expression :

$$[P'] = [P^-] + [HP] + [H_2P^+] \tag{3.37}$$

This can be rewritten in a different manner :

$$[P'] = 1 + [H^+] \cdot K_{HP} + [H^+]^2 \cdot K_{H_2P^+} \cdot K_{HP} \tag{3.38}$$

Therefore, the fraction of picolinic acid in the form P^- can be represented by the expression $1/[P']$. The appropriate conditional formation constants can then be obtained (Table 3.4b) as shown below.

$$\beta_1' = \beta_1 \cdot \alpha_{P^-} = \frac{[MgP^+]}{[Mg^{2+}][P']} \tag{3.39}$$

$$\beta_2' = \beta_2 \cdot (\alpha_{P^-})^2 = \frac{[MgP_2]}{[Mg^{2+}][P']^2} \tag{3.40}$$

The expression for the species distribution of magnesium can now be derived.

$$\alpha_{Mg^{2+}} = \frac{[Mg^{2+}]}{C_{Mg}} \tag{3.41}$$

$$\alpha_{Mg^{2+}} = \frac{[Mg^{2+}]}{[Mg^{2+}] + [MgP^+] + [MgP_2]} \tag{3.42}$$

REACTION	log K_{LIT} (25°C, O M)	log K_{NEW} (25°C, O.2 M)	FRACTION OF SPECIES (pH=6) (25°C, O.2 M)
$H^+ + P^- \Leftrightarrow HP$	5.39	5.14	$\alpha_{P^-} = 0.88$
$H^+ + HP \Leftrightarrow H_2P^+$	1.01	1.01	$\alpha_{HP} = 0.12$
			$\alpha_{H_2P^+} = 1.24 \times 10^{-6}$

REACTION	log βLIT (25°C, 0 M)	log βNEW (25°C, 0.2 M)	log β' (pH=6) (25°C, 0.2 M)
$Mg^{2+} + P^- \Leftrightarrow MgP^+$	2.58	2.08	2.03
$Mg^{2+} + 2P^- \Leftrightarrow MgP_2$	3.95	3.20	3.09

Table 3.4. (a) Top - Protonation values for Picolinic acid
(b) Bottom - Complexation values for Mg-Picolinic acid

$$\alpha_{Mg^{2+}} = \frac{[Mg^{2+}]}{[Mg^{2+}] + (\beta_1' \cdot [Mg^{2+}] \cdot [P']) + (\beta_2' \cdot [Mg^{2+}] \cdot [P']^2)} \quad (3.43)$$

$$\alpha_{Mg^{2+}} = \frac{1}{1 + \beta_1' \cdot [P'] + \beta_2' \cdot [P']^2} \quad (3.44)$$

Because of the squared term, this expression cannot be taken further as was the case in the EDTA derivation. Therefore, in order to solve this equation, an iterative process must be used. To facilitate these calculations, a computer program was written for this purpose. This program appears in appendix B2. The program uses the literature formation constants and calculates the following :

a) the β and K values at a specified I

b) the species distribution of picolinic acid at the specified pH

c) the β' values for the magnesium-picolinic acid complexes

d) the fractions of Mg^{2+}, MgP^+ and MgP_2 in a solution of a given C_{Mg} and C_P

The specificity experiments for Mg^{2+} in the presence of picolinic acid will be examined using the P-Phenylglycine and P-Picolinic acid immobilized ligand systems. These studies will determine if the immobilized ligands are specific for Mg^{2+} in the presence of its positive and neutral metal-ligand complexes (MgP^+ and MgP_2).

3.2.2.1 P-PICOLINIC ACID AS IMMOBILIZED LIGAND

Note that in this case, picolinic acid is both the immobilized ligand and the ligand dissolved in solution.

The results for the specificity of P-Picolinic acid for Mg^{2+} ions in the presence of its positive and neutral complexes (Figure 3.20) appears to be successful. The agreement between standard and sample responses are good. However, closer examination of the data reveals that there is a slight increase in the relative sorption error between the standards and samples as the concentration of Mg^{2+} ions in solution decreases (0.68%, 4.4%, 13%). This error could be attributed to the sorption of one or both of the magnesium-containing complexes by either the immobilized ligand or the polymer backbone. As the data in Table 3.5a indicates, the predominant complex is the positive MgP^{+} species. Since the error in the sorption values are relatively small, the presence of the neutral MgP_2 complex may be the contributing factor to this extra sorption. The sorption of neutral Mg complexes on a polystyrene-DVB polymer (amberlite XAD-2) has previously been reported [54]. In order to determine if the sorption of neutral magnesium-containing complexes is occurring, it will be necessary to study a complexing ligand that forms predominantly a neutral Mg complex in order to assign the extra sorption. This investigation will be done using the complexing ligand oxalic acid (section 3.2.3). Before doing this, the behavior of the other immobilized ligand system (P-Phenylglycine) in the presence of the added picolinic acid will be studied.

3.2.2.2 P-PHENYLGLYCINE AS IMMOBILIZED LIGAND

It is obvious from Figure 3.21 that extra sorption is a major problem in this system. Again, as the concentration of complexing ligand, picolinic acid, added to the solution decreases, the agreement between standard and sample

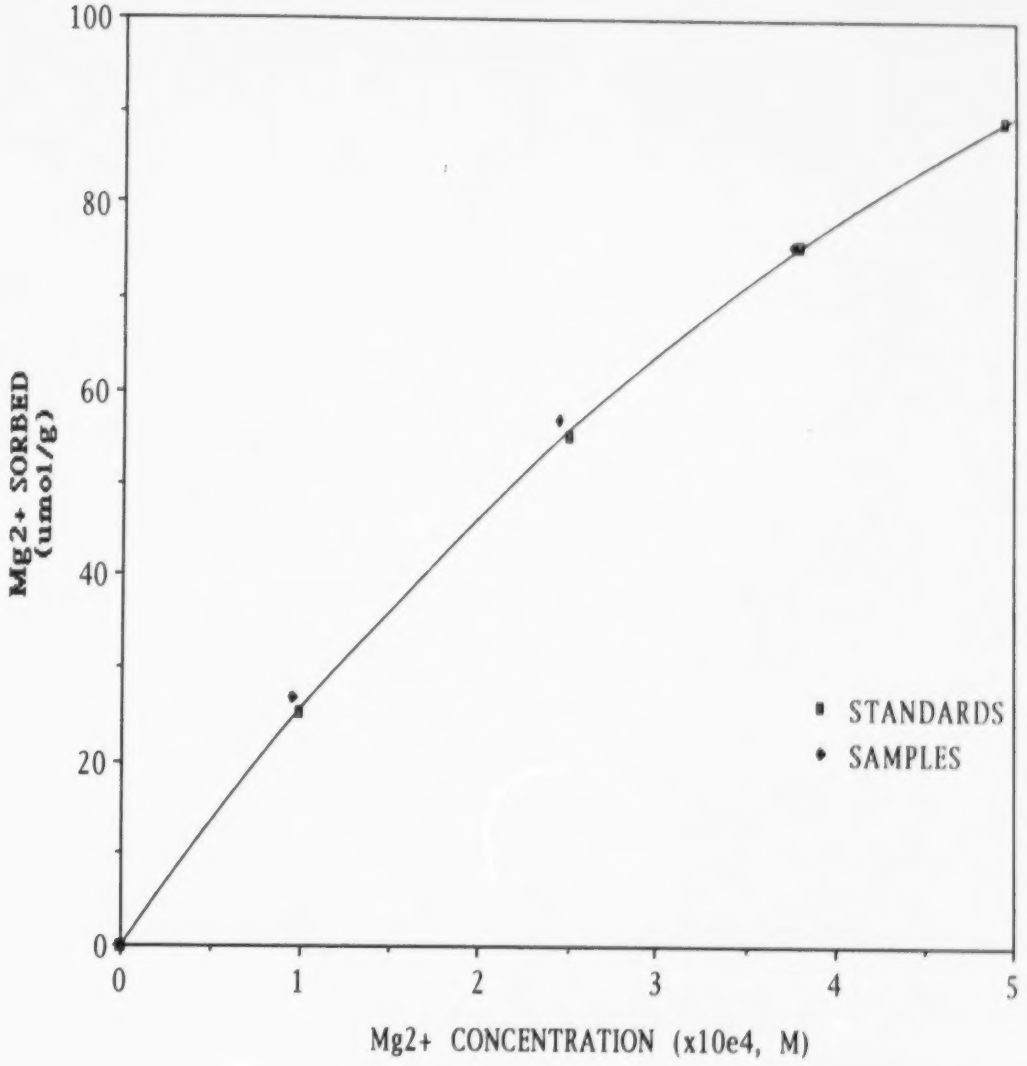


Figure 3.20. PICOLINIC ACID SPECIFICITY : P-PICOLINIC ACID

SAMPLES: 4.9×10^{-4} M Mg^{2+} ; X M Picolinic acid. STANDARDS: Y M Mg^{2+} . BOTH: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load solution, 15 min; Elute; Collect in a vial; Analyze.

[Mg] TOTAL ADDED ($x10^{-4}$ M)	[Picolinic] TOTAL ADDED ($x10^{-3}$ M)	pH	α Mg^{2+}	α MgP^{+}	α MgP_2	pMg CALC.	pMg EXPT.
4.94	30.00	6.00	0.192	0.602	0.206	4.02	3.96
4.94	9.00	6.00	0.495	0.458	0.046	3.61	3.59
4.94	3.00	6.00	0.760	0.232	0.008	3.43	3.42

[Mg] TOTAL ADDED ($x10^{-4}$ M)	[Picolinic] TOTAL ADDED ($x10^{-3}$ M)	pH	α Mg^{2+}	α MgP^{+}	α MgP_2	pMg CALC.	pMg EXPT.
5.00	20.00	6.00	0.282	0.585	0.133	3.85	3.10
5.00	8.00	6.00	0.528	0.433	0.039	3.58	3.14
5.00	4.00	6.00	0.701	0.286	0.013	3.46	3.16

Table 3.5. (a) Top - Mg^{2+} specificity in the presence of Mg-Picolinic acid complexes using the P-Picolinic acid system
(b) Bottom - Mg^{2+} specificity in the presence of Mg-Picolinic acid complexes using the P-Phenylglycine system

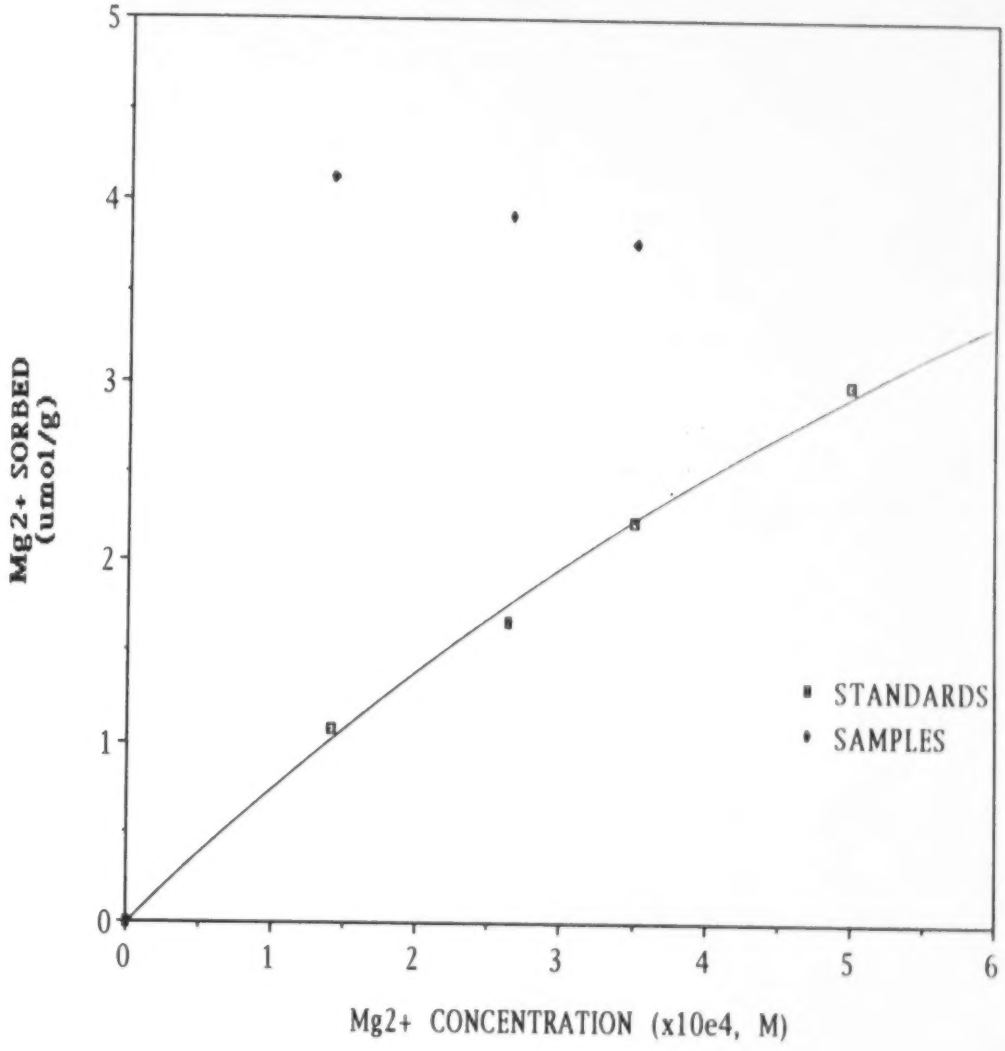


Figure 3.21. PICOLINIC ACID SPECIFICITY : P-PHENYLGLYCINE

SAMPLES: $5x10^{-4}$ M Mg^{2+}; X M Picolinic acid. STANDARDS: Y M Mg^{2+}. BOTH: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=6.00. PROCEDURE: Water rinse, 2 min; Load solution, 10 min; Elute; Inject standards.

values becomes closer (Table 3.5b). As noted in the P-Picolinic acid case, this extra sorption can be attibuted to either MgP^+ or MgP_2 or both of the species. Extra sorption of these species could be occurring by the immobilized ligand or directly onto the polymer backbone.

It is interesting to compare the Y-axis values for this system with those in the P-Picolinic acid system (Figure 3.20). The values obtained in the P-Picolinic acid system are about 30 times larger than the values obtained with this system. Therefore, an extra sorption of 2 or 3 μmol/g in this system is dramatic whereas the same situation in the other system is barely noticed. The extra sorption in that case becomes insignificant when the Mg^{2+} sorption is high enough. This situation is similar to the blank or hold-up volume problem in the CPG-Phenanthroline system. When the capacity was increased, the blank contribution was drastically reduced.

3.2.3 OXALIC ACID AS SOLUBLE LIGAND

In this section, the following notations are used :

a) IONS IN SOLUTION : Mg^{2+}, Ox^{2-}

b) COMPLEXES IN SOLUTION : MgOx, $MgOx_2^{2-}$

c) FORMATION CONSTANTS : β_1, β_2

d) FRACTION OF SPECIES IN THE GIVEN FORM : α_{Mg2+} , α_{Ox2-}

e) CONDITIONAL FORMATION CONSTANTS : β_1', β_2'

f) TOTAL CONCENTRATIONS ADDED TO SOLUTION : C_{Mg}, C_{Ox}

g) TOTAL CONCENTRATION OF UNCOMPLEXED OXALIC ACID : $[Ox']$

Oxalic acid (Ox) forms two complexes with Mg^{2+} : MgOx and $MgOx_2^{2-}$. The formation expressions for these two complexes as given in Smith and Martell [51] are as follows :

$$Mg^{2+} + Ox^{2-} \Leftrightarrow MgOx \qquad \beta_1 = \frac{[MgOx]}{[Mg^{2+}][Ox^{2-}]} \tag{3.45}$$

$$Mg^{2+} + 2Ox^{2-} \Leftrightarrow MgOx_2^{2-} \qquad \beta_2 = \frac{[MgOx_2^{2-}]}{[Mg^{2+}][Ox^{2-}]^2} \tag{3.46}$$

It is evident that this system is similar to the Mg-Picolinic acid system. In fact, the derivations for the equations are the same. Therefore, many of the steps which lead to the following equations are left out in this section but can be found in the Mg-Picolinic acid section. The formation values are in Table 3.6a and the conditional formation values are in Table 3.6b.

The conditional formation constants for this system are as follows :

$$\beta_1' = \beta_1 \cdot \alpha_{Ox^{2-}} = \frac{[MgOx]}{[Mg^{2+}][Ox']} \tag{3.47}$$

$$\beta_2' = \beta_2 \cdot (\alpha_{Ox^{2-}})^2 = \frac{[MgOx_2^{2-}]}{[Mg^{2+}][Ox']^2} \tag{3.48}$$

The expression for the species distribution of magnesium is then obtained :

$$\alpha_{Mg^{2+}} = \frac{1}{1 + \beta_1' \cdot [Ox'] + \beta_2' \cdot [Ox']^2} \tag{3.49}$$

A similar computer program to that used in the Mg-Picolinic acid case is used to solve this equation. This program is in appendix B3. The program uses the literature formation constants and calculates the following :

REACTION	log K_{LIT} (25°C, O.1 M)	log K_{NEW} (25°C, O.2 M)	FRACTION OF SPECIES (pH=6) (25°C, O.2 M)
$H^+ + Ox^{2-} \Leftrightarrow HOx^-$	3.82	3.76	$\alpha_{Ox^{2-}} = 0.99$
$H^+ + HOx^- \Leftrightarrow H_2Ox$	1.04	1.02	$\alpha_{HOx^-} = 0.01$
			$\alpha_{H_2Ox} = 6.03 \times 10^{-8}$

REACTION	log βLIT (25°C, 0.1 M)	log βNEW (25°C, 0.2 M)	log β' (pH=6) (25°C,0.2M)
$Mg^{2+} + Ox^{2-} \Leftrightarrow MgOx$	2.76	2.60	2.60
$Mg^{2+} + 2Ox^{2-} \Leftrightarrow MgOx_2^{2-}$	4.24	4.08	4.08

Table 3.6. (a) Top - Protonation values for Oxalic acid
(b) Bottom - Complexation values for Mg-Oxalic acid

a) the β and K values at a specified I

b) the species distribution of oxalic acid at the specified pH

c) the β' values for the magnesium-oxalic acid complexes

d) the fractions of Mg^{2+}, MgOx and $MgOx_2^{2-}$ in a solution of a given C_{Mg} and C_{Ox}

The specificity experiment for Mg^{2+} in the presence of oxalic acid will be examined using the P-Picolinic acid system only. An understanding of the excess sorption is needed before any further investigations using the P-Phenylglycine immobilized ligand can be performed. The following study will determine if the immobilized ligand is specific for Mg^{2+} in the presence of its neutral metal-ligand complex (MgOx).

3.2.3.1 P-PICOLINIC ACID

So far, the immobilized ligands have been investigated for their specificity in the presence of anionic (MgY^{2-}, $MgHY^{-}$) and cationic (MgP^{+}) Mg-containing complexes. To complete the specificity study of an immobilized ligand for Mg^{2+}, the soluble complexing ligand oxalic acid is used. Oxalic acid is also chosen because of the large fraction of neutral complex (MgP_2) that forms under the given experimental conditions. Therefore, any excess sorption due to the presence of neutral complexes will be amplified. As seen in Table 3.7, oxalic acid satisfies the condition of having a large fraction of neutral species present under the experimental conditions used.

At all combinations of sample and standard values, extra sorption is obtained (Figure 3.22). The data suggests that the extra sorption is due to the

[Mg] TOTAL ADDED ($x10^{-4}$ M)	[Oxalic] TOTAL ADDED ($x10^{-3}$ M)	pH	α Mg^{2+}	α MgOx	α $MgOx_2^{2-}$	pMg CALC.	pMg EXPT.
4.94	7.00	6.00	0.241	0.634	0.125	3.92	3.79
4.94	2.50	6.00	0.511	0.459	0.031	3.60	3.53
4.94	0.90	6.00	0.758	0.236	0.006	3.43	3.38

Table 3.7. Mg^{2+} specificity in the presence of Mg-Oxalic acid complexes using the P-picolinic acid system

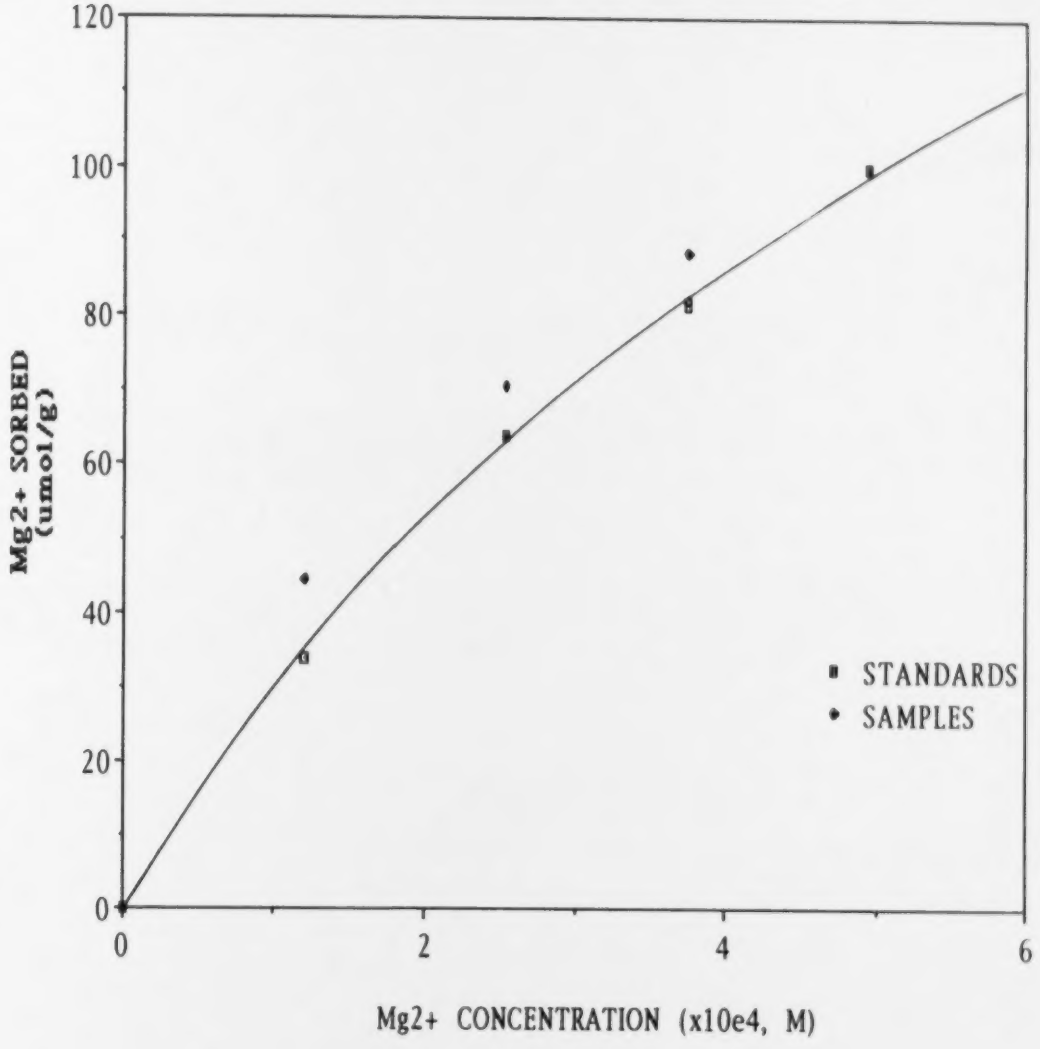


Figure 3.22. OXALIC ACID SPECIFICITY : P-PICOLINIC ACID

SAMPLES: 4.9×10^{-4} M Mg^{2+}; X M Oxalic acid. STANDARDS: Y M Mg^{2+}. BOTH: 0.2 M ionic strength ($NaClO_4$); 0.01 M HEPES buffer ; pH=6.00. PROCEDURE: Water rinse, 2 min; Load solution, 15 min; Elute; Collect in a vial; Analyze.

sorption of neutral complexes. Again, the relative error in sorption increases as the fraction of neutral complex increases.

3.2.4 SPECIFICITY SUMMARY

The specificity experiments were performed using the immobilized ligands P-Picolinic acid and P-Phenylglycine. It was found through studies involving the complexing ligand EDTA that both systems showed excellent specificity for Mg^{2+} ions in the presence of anionic Mg-containing complexes. In the studies involving soluble picolinic acid and oxalic acid, it was found that extra sorption was occurring. When the extent of sorption of Mg^{2+} ions was high enough, the extra sorption contribution was minimized. On the assumption that Mg-ligand complexes are being sorbed onto the polymer backbone, while the Mg^{2+} species is being sorbed by the immobilized ligand, this implies that if the signal obtained for the sorption of Mg^{2+} ions can be increased, that the extra sorption signal will be less significant.

To prove that the extra sorption is due to the sorption of neutral complexes onto the polymer backbone of the immobilized ligand, it would be necessary to perform sorption studies with the polymer support alone (without the immobilized ligand). This is beyond the focus of this work since quantification of the source of error is not necessary. Rather, the observation alone requires that the design of future immobilized ligands address the discovered deficiencies. With this in mind, it was decided to take the P-Picolinic acid system and perform some additional characterizing experiments. This information could be useful in future designs of immobilized ligands.

3.3 ADDITIONAL STUDIES

From the last section, it was evident that there were variables that affected the complexation between Mg^{2+} ions and ligands in solution. Examples of these variables are ionic strength and pH. The ionic strength of the solution affects the activities of the individual ions which affects the conditional formation constant. Also, the pH of the solution affects the distribution or fraction of particular species which, in turn, again affects the conditional formation constant. Since the solutions that this method could be applied to, such as biological fluids, have a range of pH and ionic strength values, it was decided to perform these types of studies on the P-Picolinic acid immobilized ligand system.

3.3.1 EFFECT OF IONIC STRENGTH

As seen from Figure 3.23, at constant pH, as the ionic strength of the sample solution is increased the sorption of Mg^{2+} by the immobilized ligand decreases. If this situation occurred in bulk solution - meaning that if soluble instead of immobilized picolinic acid was used to complex Mg^{2+} - then the following equation could be used to describe the bulk solution properties :

$$[Mg^{2+}] = \frac{[MgP^+]}{\beta_0} \frac{K^0 \cdot a_{H^+}}{[HP]} \frac{\gamma_-}{\gamma_{2+}} \qquad (3.50)$$

where β_0 is the formation constant for MgP^+; K^0 is the protonation constant for HP; a_{H+} is the hydrogen ion activity, and; γ_- and γ_{2+} are the activity coefficients for the species P^- and Mg^{2+}, respectively. When the ionic strength is increased,

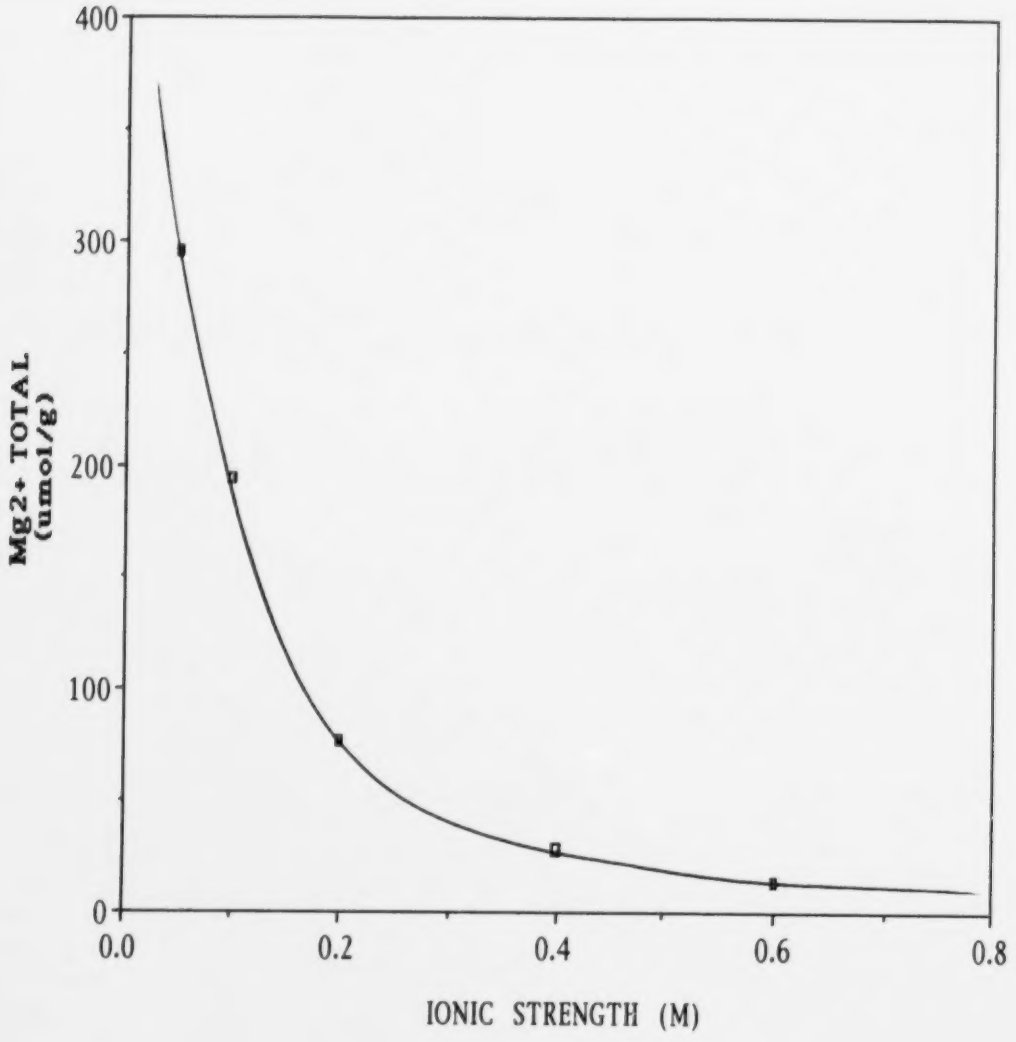


Figure 3.23. EFFECT OF IONIC STRENGTH: P-PICOLINIC ACID

SAMPLES: 4.9×10^{-4} M Mg^{2+}; X M ionic strength ($NaClO_4$); 0.01 M HEPES buffer; pH=7.00. PROCEDURE: Water rinse, 2 min; Load sample, 8 min; Elute; Collect in a vial; Analyze.

the activity coefficients decrease. In fact, the γ_{2+} term will decrease more than the γ_{-} term. As a result, the $[Mg^{2+}]$ term will increase when the ionic strength is increased.

However, this study involves an immobilized ligand. Therefore, the bulk solution treatment does not apply to this situation since the surface exhibits its own charge. For the sorption of Ca^{2+} on CPG-Oxine, it was possible to theoretically evaluate the effect of ionic strength using a modified ionizable surface group site-binding model [10]. In that study, it was possible to evaluate the formation constant for the bound oxine group and the calcium ion. In the present study, similar to a study of the sorption of Mg^{2+} on XAD-Oxine [55], this model treatment for immobilized picolinic acid cannot be used due to an inability to accurately measure the surface charge density in this system. Even though this system could not be quantitated, qualitatively, the results obtained for the relationship between ionic strength and metal ion sorption for the CPG-Oxine and XAD-Oxine were similar to those obtained here for the P-Picolinic acid-Mg^{2+} system.

3.3.2 EFFECT OF pH

In the CPG-Oxine study [10], at constant ionic strength, there was found to be a direct relationship between the distribution constant, λ_o, and the fraction of bound oxine which was ionized. This resulted in a similar plot of λ_o versus pH as is seen in Figure 3.24. Recall from equation (1.1), that λ_o is proportional to Mg^{2+} sorbed. However, a modified ionizable surface group site-binding model cannot be used to quantitate this P-Picolinic acid system for the same reason as given above in the ionic strength discussion. Qualitatively, it is

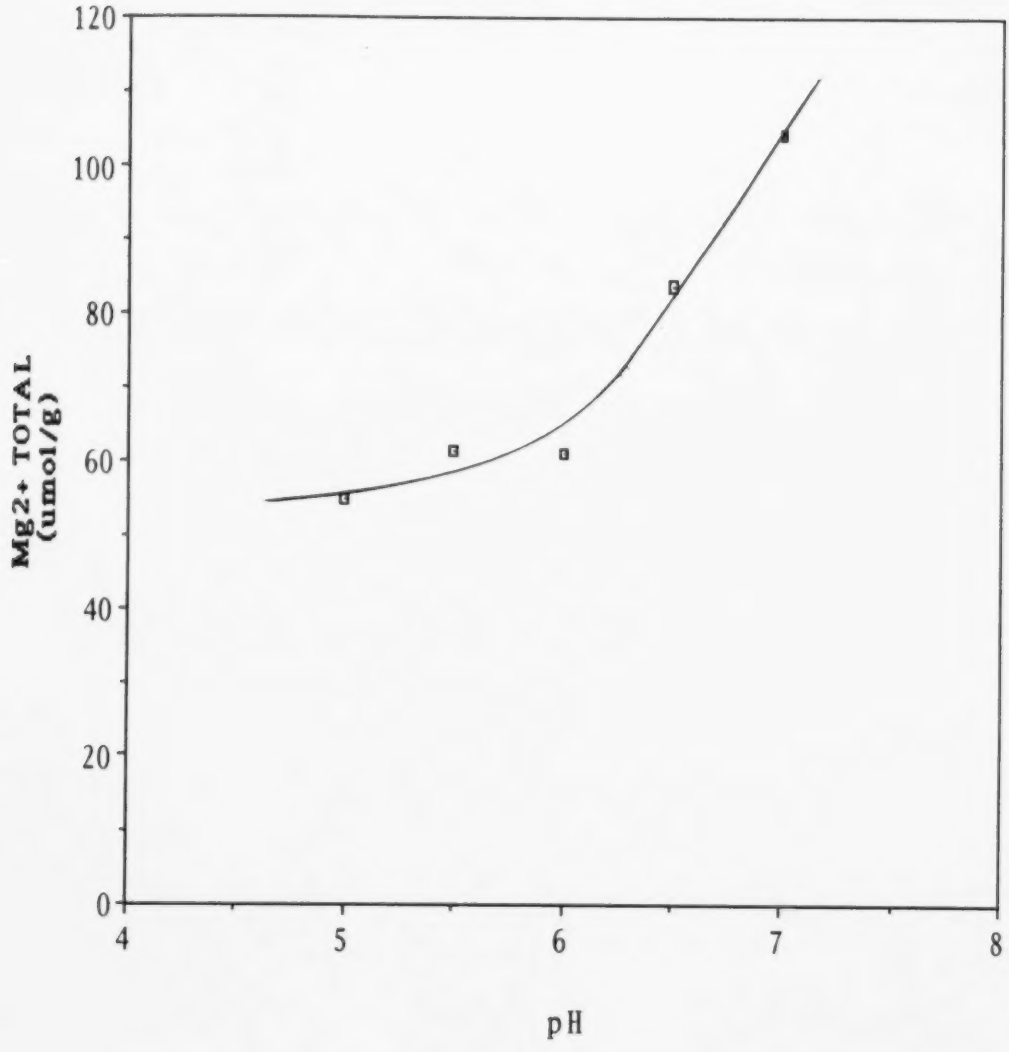


Figure 3.24. EFFECT OF pH : P-PICOLINIC ACID

SAMPLES: 4.9×10^{-4} M Mg^{2+}; 0.2 M ionic strength($NaClO_4$); 0.01 M HEPES buffer; pH=X. PROCEDURE: Water rinse, 2 min; Load sample, 8 min; Elute; Collect in a vial; Analyze.

recognized that at higher pH values, the fraction of $(P^-)_R$ increases. This being the case, the observation that the sorption of Mg^{2+} ions increases is expected even though the exact nature of the system is not able to be calculated.

3.3.3 SUMMARY

The ionic strength and pH studies provided some valuable information. It was found that the sorption of Mg^{2+} ions by the P-Picolinic acid immobilized ligand system was dependent on the conditions of the tested solution. This means that there is a requirement for exact matching of standard and sample solution pH and ionic strength in order to obtain accurate results for the concentration of uncomplexed Mg^{2+} ions in solution.

Ideally, the variables that affect the sorption of Mg^{2+} ions would yield flat or horizontal response curves meaning that the results obtained would be independent of the solution conditions. Examination of the ionic strength study (equation 3.50) reveals that it would be necessary to select an immobilized ligand that resulted in all of the activity coefficients being canceled out. In effect, the sorption of Mg^{2+} ions would then be independent of ionic strength. In the pH study, there is no way of canceling out the effect of changing the a $_{H+}$ term. However, instead of having a contribution to the concentration of P^- from the changing fraction of P^-, it is desirable to have the fraction of P^- constant over a given pH range. This would involve the selection of a ligand with the appropriate protonation values.

As was the case throughout, the observed results from this immobilized ligand will influence the design of future immobilized ligands.

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APPENDICES

This section contains :

Appendix A : Data tables for the figures in the thesis

Appendix B : Computer programs used in the specificity studies

APPENDIX A

Table A1. Cu^{2+} WASH STUDY : CPG-PHENANTHROLINE. Data for Figure 2.9.

WASH TIME (MINUTES)	Cu^{2+} SORBED (μmol/g)
0.00	8.90
2.00	1.48
4.00	1.52
6.00	1.54
8.00	1.53

Table A2. Cu^{2+} LOAD STUDY : CPG-PHENANTHROLINE. Data for Figure 2.10.

LOAD TIME (MINUTES)	Cu^{2+} SORBED (μmol/g)
0.25	0.55
0.50	0.71
2.00	0.83
4.00	0.87
6.00	0.84

Table A3. Cu^{2+} CONCENTRATION STUDY : CPG-PHENANTHROLINE.
Data for Figure 2.11.

SAMPLE CONCENTRATION ($x10^{-3}$ M)	Cu^{2+} SORBED (μmol/g)
0.16	0.62
0.31	0.87
0.63	1.01
1.26	1.15
1.57	1.17
1.89	1.15

Table A4. LOAD STUDY : CPG-OXINE.
Data for Figure 3.1.

LOAD TIME (MINUTES)	Mg^{2+} TOTAL (μmol/g)
0.050	0.357
0.083	0.512
0.167	0.560
0.250	0.640
0.500	0.709
1.000	0.723
2.000	0.712
4.000	0.723
8.000	0.693
15.000	0.628
60.000	0.629

Table A5. ISOTHERM STUDY : CPG-OXINE.
Data for Figure 3.2.

Mg^{2+} CONCENTRATION ($x10^{-5}$ M)	INTEGRATED PEAK AREA (ABSORBANCE x TIME UNITS)
0	0
0.82	0.036
2.47	0.106
4.11	0.155
8.23	0.246

Table A6. LOAD STUDY : CPG-PHENANTHROLINE.
Data for Figure 3.3. (Hold-up Volume = 17.1 μL).

LOAD TIME (MINUTES)	Mg^{2+} SORBED (μmol/g)
0.083	0.027
0.167	0.089
0.250	0.099
1.000	0.135
2.000	0.159
4.000	0.176
8.000	0.182
15.00	0.226
30.00	0.284
60.00	0.375

Table A7. ISOTHERM AND BLANK STUDY : CPG-PHENANTHROLINE. Data for Figure 3.4.

Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} TOTAL (μmol/g)	Mg^{2+} BLANK (μmol/g)
0	0	0
0.25	0.111	0.079
0.41	0.183	0.129
0.82	0.378	0.270
2.06	0.910	0.642
4.11	1.75	1.26
8.23	3.29	2.46

Table A8. ISOTHERM STUDY : CPG-PHENANTHROLINE. Data for Figure 3.5.

Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
0	0
0.25	0.032
0.41	0.055
0.82	0.108
2.06	0.267
4.11	0.492
8.23	0.829

Table A9. LOAD STUDY : P-PHENANTHROLINE.
Data for Figure 3.6 (Hold-up volume = 24.0 μL).

LOAD TIME (MINUTES)	Mg^{2+} SORBED (μmol/g)
0.083	1.07
0.167	1.38
0.250	1.80
0.500	3.83
1.000	5.88
2.000	8.89
4.000	12.1
8.000	14.2
15.000	15.0
60.000	15.0

Table A10. ISOTHERM AND BLANK STUDY : P-PHENANTHROLINE.
Data for Figure 3.7.

Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} TOTAL (μmol/g)	Mg^{2+} BLANK (μmol/g)
0	0	0
0.41	18.1	0.371
0.82	33.8	0.620
2.06	81.4	1.54
4.11	144	2.75
8.23	231	6.06

Table A11. ISOTHERM STUDY : P-PHENANTHROLINE.
Data for Figure 3.8.

Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
0	0
0.41	17.7
0.82	33.2
2.06	79.9
4.11	142
8.23	225

Table A12. LOAD STUDY : P-PHENYLGLYCINE.
Data for Figure 3.9.

LOAD TIME (MINUTES)	Mg^{2+} TOTAL (μmol/g)
1.00	0.317
2.00	0.342
4.00	0.374
8.00	0.376
15.00	0.413
30.00	0.458
60.00	0.564

Table A13. PRECONDITIONING LOAD STUDY : P-PHENYLGLYCINE. Data for Figure 3.10.

LOAD TIME (MINUTES)	Mg^{2+} TOTAL (μmol/g)
2.00	0.043
5.00	0.097
10.00	0.151
15.00	0.172
30.00	0.312
60.00	0.562

Table A14. FILTERED PRECONDITIONING STUDY : P-PHENYLGLYCINE. Data for Figure 3.11.

LOAD TIME (MINUTES)	Mg^{2+} TOTAL (μmol/g)
2.00	0.017
5.00	0.017
15.00	0.018
30.00	0.016
60.00	0.016

Table A15. LOAD STUDY : P-PHENYLGLYCINE.
Data for Figure 3.12 (Hold-up volume = 15.7 μL).

LOAD TIME (MINUTES)	Mg^{2+} SORBED (μmol/g)
1.00	1.46
2.00	1.67
5.00	1.74
10.00	1.75
15.00	1.75
25.00	1.75
60.00	1.76

Table A16. ISOTHERM AND BLANK STUDY : P-PHENYLGLYCINE.
Data for Figure 3.13.

Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} TOTAL (μmol/g)	Mg^{2+} BLANK (μmol/g)
0	0	0
0.50	0.641	0.148
1.00	0.918	0.228
5.00	5.08	1.63
7.50	7.20	2.47
10.00	9.13	3.40

Table A17. ISOTHERM STUDY : P-PHENYLGLYCINE.
Data for Figure 3.14.

Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
0	0
0.50	0.493
1.00	0.690
5.00	3.46
7.50	4.73
10.00	5.73

Table A18. LOAD STUDY : P-PICOLINIC ACID.
Data for Figure 3.15 (Hold-up volume = 43.2 μL).

LOAD TIME (MINUTES)	Mg^{2+} SORBED (μmol/g)
1.000	13.7
2.000	19.3
4.000	24.2
8.000	27.1
15.000	28.3
30.000	28.1

Table A19. TYPICAL CALIBRATION CURVE.
Data for Figure 3.16.

Mg^{2+} CONCENTRATION ($x10^{-5}$ M)	ABSORBANCE
0	0
2.06	0.204
4.11	0.414
6.17	0.623
8.23	0.811
10.29	1.014

Table A20. ISOTHERM STUDY : P-PICOLINIC ACID.
Data for Figure 3.17 (Hold-up volume = 43.2 μL).

Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
0	0
0.33	8.47
0.66	17.3
0.99	24.3
2.96	62.4
4.94	97.0
6.91	122

Table A21. Mg-EDTA SPECIFICITY STUDY : P-PICOLINIC ACID. Data for Figure 3.18 (Hold-up volume = 43.2 μL).

SOLUTION	Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
STANDARD	0	0
STANDARD	0.99	23.6
STANDARD	2.47	52.6
STANDARD	3.70	72.1
STANDARD	4.94	87.2
SAMPLE	0	0
SAMPLE	1.03	23.6
SAMPLE	2.50	50.5
SAMPLE	3.72	72.8

Table A22. Mg-EDTA SPECIFICITY STUDY : P-PHENYLGLYCINE. Data for Figure 3.19 (Hold-up volume = 15.7 μL).

SOLUTION	Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
STANDARD	0	0
STANDARD	1.26	1.05
STANDARD	2.62	1.92
STANDARD	3.71	2.51
STANDARD	5.00	3.17
SAMPLE	0	0
SAMPLE	1.27	1.08
SAMPLE	2.62	1.87
SAMPLE	3.71	2.56

Table A23. Mg-PICOLINIC ACID SPECIFICITY : P-PICOLINIC ACID. Data for Figure 3.20 (Hold-up volume = 43.2 μL).

SOLUTION	Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
STANDARD	0	0
STANDARD	0.99	25.1
STANDARD	2.49	55.0
STANDARD	3.79	75.6
STANDARD	4.94	89.2
SAMPLE	0	0
SAMPLE	0.95	26.8
SAMPLE	2.44	56.8
SAMPLE	3.75	75.7

Table A24. Mg-PICOLINIC ACID SPECIFICITY : P-PHENYLGLYCINE. Data for Figure 3.21 (Hold-up volume = 15.7 μL).

SOLUTION	Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
STANDARD	0	0
STANDARD	1.41	1.08
STANDARD	2.64	1.66
STANDARD	3.51	2.21
STANDARD	5.00	2.99
SAMPLE	0	0
SAMPLE	1.41	4.11
SAMPLE	2.64	3.90
SAMPLE	3.51	3.76

Table A25. Mg-OXALIC ACID SPECIFICITY : P-PICOLINIC ACID. Data for Figure 3.22 (Hold-up volume = 43.2 μL).

SOLUTION	Mg^{2+} CONCENTRATION ($x10^{-4}$ M)	Mg^{2+} SORBED (μmol/g)
STANDARD	0	0
STANDARD	1.19	33.8
STANDARD	2.52	63.5
STANDARD	3.74	81.4
STANDARD	4.94	99.8
SAMPLE	0	0
SAMPLE	1.19	44.3
SAMPLE	2.52	70.2
SAMPLE	3.74	88.2

Table A26. IONIC STRENGTH STUDY : P-PICOLINIC ACID. Data for Figure 3.23.

IONIC STRENGTH (M)	Mg^{2+} TOTAL (μmol/g)
0.05	296
0.10	195
0.20	77.2
0.40	28.0
0.60	14.5

Table A27. pH STUDY : P-PICOLINIC ACID.
Data for Figure 3.24.

pH	Mg^{2+} TOTAL (μmol/g)
5.00	54.7
5.50	61.2
6.00	61.1
6.50	83.6
7.00	104

APPENDIX B

COMPUTER PROGRAM B1 : Mg-EDTA SPECIFICITY

```
REM  THIS PROGRAM IS USED TO DETERMINE THE DISTRIBUTION OF
REM  Mg SPECIES IN THE PRESENCE OF THE COMPLEXING LIGAND,
REM  EDTA.

INPUT       "The pH of the solution = ";pH
LPRINT      "Mg-Na-EDTA"
LPRINT      "The pH of the solution = ";pH

REM  THE FOLLOWING ARE THE LOG K VALUES FOR THE GIVEN
REM  REACTIONS

REM  Mg2+ + Y4- ↔ MgY2-
       LK(1) = 8.79
REM  MgY2- + H+ ↔ MgHY-
       LK(2) = 3.85
REM  Y4- + H+ ↔ HY3-
       LK(3) = 10.24
REM  HY3- + H+ ↔ H2Y2-
       LK(4) = 6.16
REM  H2Y2- + H+ ↔ H3Y-
       LK(5) = 2.66
REM  H3Y- + H+ ↔ H4Y
       LK(6) = 2!
REM  H4Y + H+ ↔ H5Y+
       LK(7) = 1.5
REM  Na+ + Y4- ↔ NaY3-
       LK(8) = 1.66
REM  Mg2+ + HY3- ↔ MgHY-
       LK(9) = LK(1) + LK(2) - LK(3)

REM  CONVERSION OF LOG K VALUES TO K VALUES

FOR i = 1 TO 9
K(i) = 10^LK(i)
NEXT i
```

```
REM  CALCULATION OF ACTIVITY COEFFICIENTS USING THE IONIC
REM  STRENGTH QUOTED FOR LITERATURE K VALUES.
REM  THE SUBROUTINE IS THE DAVIES EQUATION ( SEE END OF
REM  PROGRAM )

IS = .1
Z=0:GOSUB 5000:AC10=AC
Z=1:GOSUB 5000:AC11=AC
Z=2:GOSUB 5000:AC12=AC
Z=3:GOSUB 5000:AC13=AC
Z=4:GOSUB 5000:AC14=AC

REM  CALCULATION OF THE ACTIVITY COEFFICIENTS USING THE
REM  DESIRED EXPERIMENTAL IONIC STRENGTH

IS = .2
Z=0:GOSUB 5000:AC20=AC
Z=1:GOSUB 5000:AC21=AC
Z=2:GOSUB 5000:AC22=AC
Z=3:GOSUB 5000:AC23=AC
Z=4:GOSUB 5000:AC24=AC

REM  CONVERSION OF LITERATURE K VALUES TO THE K VALUES AT
REM  THE DESIRED IONIC STRENGTH FOR EDTA

K(3) = K(3) * AC13 * AC24 / AC14 / AC23
K(4) = K(4) * AC12 * AC23 / AC13 / AC22
K(5) = K(5) * AC11 * AC22 / AC12 / AC21
K(6) = K(6) * AC10 * AC21 / AC11 / AC20
K(7) = K(7) * AC11 * AC20 / AC10 / AC21

H = 10^(-pH)

REM  THE CALCULATION OF THE SPECIES DISTRIBUTION OF EDTA

D = 1 + K(3)*H + K(3)*K(4)*H^2 + K(3)*K(4)*K(5)*H^3 +
    K(3)*K(4)*K(5)*K(6)*H^4+K(3)*K(4)*K(5)*K(6)*K(7)*H^5
B4 = 1/D
B3 = K(3)*H/D
B2 = K(3)*K(4)*H^2/D
B1 = K(3)*K(4)*K(5)*H^3/D
B0 = K(3)*K(4)*K(5)*K(6)*H^4/D
BP = K(3)*K(4)*K(5)*K(6)*K(7)*H^5/D
```

```
REM  CONVERSION OF LITERATURE K VALUES TO THE K VALUES AT
REM  THE DESIRED IONIC STRENGTH FOR Mg AND Na

K(1) = K(1) * AC24 / AC14
K(8) = K(8) * AC13 * AC21 * AC24 / AC11 / AC14 / AC23
K(9) = K(9) * AC11 * AC22 * AC23 / AC12 / AC13 / AC21

REM  CONVERSION OF THE NEW K VALUES TO LOG K VALUES

LK(1) = LOG(K(1))/LOG(10)
FOR i = 3 TO 9
LK(i) = LOG(K(i))/LOG(10)
NEXT i

LPRINT "LOG K VALUES AT 0.2 M IONIC STRENGTH"
LPRINT " ";LK(1),LK(3),LK(4),LK(5),LK(6),LK(7),LK(8),LK(9)

LPRINT "BETA Y4- = ";B4
LPRINT "BETA HY3- = ";B3
LPRINT "BETA H2Y2- = ";B2
LPRINT "BETA H3Y- = ";B1
LPRINT "BETA H4Y = ";B0
LPRINT "BETA H5Y+ = ";BP

REM  CALCULATION OF CONDITIONAL K VALUES FOR M-EDTA

K(1) = K(1) * B4
K(8) = K(8) * B4
K(9) = K(9) * B3

LK(1) = LOG(K(1))/LOG(10)
LK(8) = LOG(K(8))/LOG(10)
LK(9) = LOG(K(9))/LOG(10)

LPRINT "CONDITIONAL LOG K VALUES"
LPRINT " ";LK(1),LK(8),LK(9)

REM  THIS PART IS AN ITERATION PROCESS TO DETERMINE THE
REM  SPECIES DISTRIBUTION OF Mg WITH SELECTED EDTA AND
REM  NaCONCENTRATIONS.
REM  NOTE : THE [Na+] CANNOT BE SET EXACTLY UNTIL THE
REM  EXPERIMENTAL SOLUTIONS HAVE BEEN PREPARED AND THE
```

```
REM  AMOUNT OF Na ADDED CALCULATED.  THIS DOES NOT CAUSE
REM  ANY PROBLEM SINCE THE [Na+] DOES NOT HAVE A VERY
REM  SIGNIFICANT EFFECT.

INPUT "[Na], [EDTA] =";Na,Y
LPRINT "[Na] =";Na
LPRINT "[EDTA] = ";Y
INPUT "  INITIAL Mg VALUE GUESS = ";Mg

REM  THE TOAL [Mg] ADDED, CM, NEEDS TO BE TYPED IN

CM = ?
K1 = K(1)
KX = K(9)
KN = K(8)
Mg1 = Mg
100 αMg1 = Mg1/CM
Y1 = ((1/αMg1 - 1)*(1 + KN * Na) /(K1 + KX)) + CM - Mg1
IF (ABS(1 - Y1/Y)) < 1E-12 THEN GOTO 190
IF (ABS(1 - Y1/Y)) > 1E-12 THEN GOTO 140
140 Mg2 = Mg1 * Y1/Y
αMg2 = Mg2/CM
Y1 = ((1/αMg2 - 1)*(1 + KN * Na) /(K1 + KX)) + CM - Mg2
Mg1 = (Mg1 + Mg2)/2
GOTO 100
190 LPRINT "EDTA CALCULATED = ";Y1
    LPRINT "[Mg] CALCULATED = ";Mg1
    pMg = (LOG(Mg1)/LOG(10))*-1
    LPRINT "pMg THEORETICAL = ";pMg
     LPRINT "ALPHA Mg2+ CALCULATED = ";αMg1
αMgY = (K1*((Y1-CM+Mg1)/(1+KN*Na)))/(1+(K1+KX)*((Y1-
CM+Mg1)/(1+KN*Na)))
     LPRINT "ALPHA MgY2- CALCULATED = ";αMgY
αMgHY = (KX*((Y1-CM+Mg1)/(1+KN*Na)))/(1+(K1+KX)*((Y1-
CM+Mg1)/(1+KN*Na)))
     LPRINT "ALPHA MgHY- CALCULATED = ";αMgHY
END

5000 REM SUBROUTINE FOR ACTIVITY COEFFICIENT
AC=10^(-.5*Z^2*(SQR(IS)/(1+SQR(IS))-.3*IS))
RETURN
```

COMPUTER PROGRAM B2 : Mg-PICOLINIC ACID SPECIFICITY

```
REM  THIS PROGRAM IS USED TO DETERMINE THE DISTRIBUTION OF
REM  Mg SPECIES IN THE PRESENCE OF THE COMPLEXING LIGAND
REM  PICOLINIC ACID

INPUT "THE pH OF THE SOLUTION = ";pH
LPRINT "Mg-PICOLINIC ACID"
LPRINT "THE pH OF THE SOLUTION = ";pH

REM  THE FOLLOWING ARE THE LOG K VALUES FOR THE GIVEN
REM  REACTIONS

REM  Mg2+ + P- ↔ MgP+
       LB(1) = 2.58
REM  Mg2+ + 2P- ↔ MgP2
       LB(2) = 3.95
REM  H+ + P- ↔ HP
       LK(1) = 5.39
REM  H+ + HP ↔ H2P+
       LK(2) = 1.01

REM  CONVERSION OF LOG K VALUES TO K VALUES

FOR I = 1 TO 2
B(I) = 10^LB(I)
NEXT I

FOR I = 1 TO 2
K(I) = 10^LK(I)
NEXT I

REM  CALCULATION OF ACTIVITY COEFFICIENTS USING THE IONIC
REM  STRENGTH QUOTED FOR LITERATURE K VALUES.
REM  THE SUBROUTINE IS THE DAVIES EQUATION ( SEE END OF
REM  PROGRAM )

IS = 0
Z=0:GOSUB 5000:AC10=AC
Z=1:GOSUB 5000:AC11=AC
Z=2:GOSUB 5000:AC12=AC
```

```
REM  CALCULATION OF THE ACTIVITY COEFFICIENTS USING THE
REM  DESIRED EXPERIMENTAL IONIC STRENGTH

IS = .2
Z=0:GOSUB 5000:AC20=AC
Z=1:GOSUB 5000:AC21=AC
Z=2:GOSUB 5000:AC22=AC

REM  CONVERSION OF LITERATURE K VALUES TO THE K VALUES AT
REM  THE DESIRED IONIC STRENGTH FOR PICOLINIC ACID

K(1) = K(1) * AC10 * AC21 * AC21 / AC11 / AC11 / AC20
K(2) = K(2) * AC20  / AC10

H = 10^(-pH)

REM  THE CALCULATION OF THE SPECIES DISTRIBUTION OF
REM  PICOLINIC ACID

D = 1 + K(1)*H + K(1)*K(2)*H^2
B1 = 1/D
B0 = K(1)*H/D
BP = K(1)*K(2)*H^2/D

REM  CONVERSION OF LITERATURE K VALUES TO THE K VALUES AT
REM  THE DESIRED IONIC STRENGTH FOR Mg

B(1) = B(1) * AC22 / AC12
B(2) = B(2) * AC10 * AC22 * AC21 * AC21 / AC12 / AC11 / AC11 /
AC20

REM  CONVERSION OF THE NEW K VALUES TO LOG K VALUES

FOR I = 1 TO 2
LB(I) = LOG(B(I))/LOG(10)
NEXT I

LPRINT "LOG BETA VALUES AT 0.2 M IONIC STRENGTH"
LPRINT " ";LB(1),LB(2)
LPRINT
FOR I = 1 TO 2
LK(I) = LOG(K(I))/LOG(10)
NEXT I
```

```
LPRINT "LOG K VALUES AT 0.2 M IONIC STRENGTH"
LPRINT " ";LK(1),LK(2)

LPRINT "BETA P- = ";B1
LPRINT "BETA HP = ";B0
LPRINT "BETA H2P+ = ";BP

REM  CALCULATION OF CONDITIONAL K VALUES FOR Mg-P

B(1) = B(1) * B1
B(2) = B(2) * B1 * B1

LB(1) = LOG(B(1))/LOG(10)
LB(2) = LOG(B(2))/LOG(10)

LPRINT "CONDITIONAL LOG B VALUES"
LPRINT " ";LB(1),LB(2)

REM  THIS PART IS AN ITERATIVE PROCESS TO DETERMINE THF
REM  SPECIES DISTRIBUTION OF Mg WITH SELECTED PICOLINIC
REM  ACID CONCENTRATIONS

INPUT "PICOLINIC ACID CONCENTRATION = ";L

REM  NOTE THAT THE TOTAL Mg CONCENTRATION, M, NEEDS TO BE
REM  TYPED IN

M = ?
D = 1 + (B(1) * L) + (B(2) * L^2)
AM = 1/D
AML1 = B(1) * L / D
AML2 = B(2) * L^2 / D
ALT = AML1 * M + ( 2 * AML2 * M )
600 N = 1
610 IF N < 30 GOTO 630
620 IF N = 30 GOTO 710
630 LA = L -  ALT
D = 1 + (B(1) * LA) + (B(2) * LA^2)
AM = 1/D
AML1 = B(1) * LA / D
AML2 = B(2) * LA^2 / D
ALT = AML1 * M + ( 2 * AML2 *M )
N = N + 1
```

```
GOTO610
710 LPRINT " NUMBER OF ITERATIONS = ";N
LPRINT " Mg CONCENTRATION = ";M
LPRINT " PICOLINIC ACID CONCENTRATION = ";L
LPRINT " ADJUSTED PICOLINIC ACID CONCENTRATION = "; LA
Mg = M * AM
LPRINT " CALCULATED Mg CONCENTRATION = ";Mg
pMg = (LOG(Mg)/LOG(10))*-1
LPRINT " pMg THEORETICAL = ";pMg
LPRINT " ALPHA Mg2+ = "; AM
LPRINT " ALPHA MgP+ = "; AML1
LPRINT " ALPHA MgP2 = "; AML2

END

5000 REM SUBROUTINE FOR ACTIVITY COEFFICIENT
AC=10^(-.5*Z^2*(SQR(IS)/(1+SQR(IS))-.3*IS))
RETURN
```

COMPUTER PROGRAM B3 : Mg-OXALIC ACID SPECIFICITY

```
REM  THIS PROGRAM IS USED TO DETERMINE THE DISTRIBUTION OF
REM  Mg SPECIES IN THE PRESENCE OF THE COMPLEXING LIGAND
REM  OXALIC ACID

INPUT "THE pH OF THE SOLUTION = ";pH
LPRINT "Mg-Oxalic Acid"
LPRINT "THE pH OF THE SOLUTION = ";pH

REM  THE FOLLOWING ARE THE LOG K VALUES FOR THE GIVEN
REM  REACTIONS

REM  Mg2+ + Ox2- ↔ MgOx
       LB(1) = 2.76
REM  Mg2+ + 2Ox2- ↔ (MgOx2)2-
       LB(2) = 4.24
REM  H+ + Ox2- ↔ HOx-
       LK(1) = 3.82
REM  H+ + HOx- ↔ H2Ox
       LK(2) = 1.04

REM  CONVERSION OF LOG K VALUES TO K VALUES

FOR I = 1 TO 2
B(I) = 10^LB(I)
NEXT I

FOR I = 1 TO 2
K(I) = 10^LK(I)
NEXT I

REM  CALCULATION OF ACTIVITY COEFFICIENTS USING THE IONIC
REM  STRENGTH QUOTED FOR LITERATURE K VALUES.
REM  THE SUBROUTINE IS THE DAVIES EQUATION (SEE END OF
REM  PROGRAM)

IS = .1
Z=0:GOSUB 5000:AC10=AC
Z=1:GOSUB 5000:AC11=AC
Z=2:GOSUB 5000:AC12=AC
```

```
REM  CALCULATION OF THE ACTIVITY COEFFICIENTS USING THE
REM  DESIRED EXPERIMENTAL IONIC STRENGTH

IS = .2
Z=0:GOSUB 5000:AC20=AC
Z=1:GOSUB 5000:AC21=AC
Z=2:GOSUB 5000:AC22=AC

REM  CONVERSION OF LITERATURE K VALUES TO THE K VALUES AT
REM  THE DESIRED IONIC STRENGTH FOR OXALIC ACID

K(1) = K(1) * AC11 * AC22 / AC12 / AC21
K(2) = K(2) * AC10 * AC21 / AC11 / AC20

H = 10^(-pH)

REM  THE CALCULATION OF THE SPECIES DISTRIBUTION OF OXALIC
REM  ACID

D = 1 + K(1)*H + K(1)*K(2)*H^2
B2 = 1/D
B1 = K(1)*H/D
B0 = K(1)*K(2)*H^2/D

REM  CONVERSION OF LITERATURE K VALUES TO THE K VALUES AT
REM  THE DESIRED IONIC STRENGTH FOR Mg

B(1) = B(1) * AC10 * AC22 * AC22 / AC12 /AC12 / AC20
B(2) = B(2) * AC22 * AC22 / AC12 / AC12

REM  CONVERSION OF THE NEW K VALUES TO LOG K VALUES

FOR I = 1 TO 2
LB(I) = LOG(B(I))/LOG(10)
NEXT I

LPRINT "LOG BETA VALUES AT 0.2 M IONIC STRENGTH"
LPRINT " ";LB(1),LB(2)

FOR I = 1 TO 2
LK(I) = LOG(K(I))/LOG(10)
NEXT I
```

```
LPRINT "LOG K VALUES AT 0.2 M IONIC STRENGTH"
LPRINT " ";LK(1),LK(2)

LPRINT "BETA Ox2- = ";B2
LPRINT "BETA HOx- = ";B1
LPRINT "BETA H2Ox = ";B0

REM  CALCULATION OF CONDITIONAL K VALUES FOR Mg-Ox

B(1) = B(1) * B2
B(2) = B(2) * B2 * B2

LB(1) = LOG(B(1))/LOG(10)
LB(2) = LOG(B(2))/LOG(10)

LPRINT "CONDITIONAL LOG B VALUES"
LPRINT " ";LB(1),LB(2)

REM  THIS PART IS AN ITERATIVE PROCESS TO DETERMINE THE
REM  SPECIES DISTRIBUTION OF Mg WITH SELECTED OXALIC ACID
REM  CONCENTRATIONS

INPUT " Ox CONCENTRATION = "; L

REM  NOTE THAT THE TOTAL Mg CONCENTRATION, M, NEEDS TO BE
REM  TYPED IN

M = ?
D = 1 + (B(1) * L) + (B(2) * L^2)
AM = 1/D
AML1 = B(1) * L / D
AML2 = B(2) * L^2 / D
ALT = AML1 * M + ( 2 * AML2 * M )
600 N = 1
610 IF N < 30 GOTO 630
620 IF N = 30 GOTO 710
630 LA = L -  ALT
D = 1 + (B(1) * LA) + (B(2) * LA^2)
AM = 1/D
AML1 = B(1) * LA / D
AML2 = B(2) * LA^2 / D
ALT = AML1 * M + ( 2 * AML2 *M )
N = N + 1
```

```
GOTO610
710 LPRINT " NUMBER OF ITERATIONS = ";N
LPRINT " Mg CONCENTRATION = ";M
LPRINT " OXALIC ACID CONCENTRATION = ";L
LPRINT " ADJUSTED OXALIC ACID CONCENTRATION = "; LA
Mg = M * AM
LPRINT " CALCULATED Mg CONCENTRATION = ";Mg
pMg = (LOG(Mg)/LOG(10))*-1
LPRINT " pMg THEORETICAL = ";pMg
LPRINT " ALPHA Mg2+ = "; AM
LPRINT " ALPHA MgOx = "; AML1
LPRINT " ALPHA MgOx2- = "; AML2

END

5000 REM SUBROUTINE FOR ACTIVITY COEFFICIENT
AC=10^(-.5*Z^2*(SQR(IS)/(1+SQR(IS))-.3*IS))
RETURN
```